Description of **WO 02077183 (A2)**

IDENTIFICATION OF ESSENTIAL GENES IN MICROORGANISMS

Sequence Listing

The present application is being filed along with quadruplicate copies of a CD-ROM marked"Copy 1-SEQUENCE LISTING PART,""Copy 2-SEQUENCE LISTING PART," "Copy 3-SEQUENCE LISTING PART,"and"CRF"containing a Sequence Listing in electronic format. The quadruplicate copies of the CD-ROM each contain a file entitled 034VPC final. ST25. txt, created on March 15,2002, which is 181,323,311 bytes in size.

Background of the Invention

Since the discovery of penicillin, the use of antibiotics to treat the ravages of bacterial infections has saved millions of lives. With the advent of these"miracle drugs,"for a time it was popularly believed that humanity might, once and for all, be saved from the scourge of bacterial infections. In fact, during the 1980s and early 1990s, many large pharmaceutical companies cut back or eliminated antibiotics research and development. They believed that infectious disease caused by bacteria finally had been conquered and that markets for new drugs were limited.

Unfortunately, this belief was overly optimistic.

The tide is beginning to turn in favor of the bacteria as reports of drug resistant bacteria become more frequent. The United States Centers for Disease Control announced that one of the most powerful known antibiotics, vancomycin, was unable to treat an infection of the common Staphylococcus aureus (staph). This organism is commonly found in our environment and is responsible for many nosocomial infections. The import of this announcement becomes clear when one considers that vancomycin was used for years to treat infections caused by Staphylococcus species as well as other stubborn strains of bacteria. In short, bacteria are becoming resistant to our most powerful antibiotics. If this trend continues, it is conceivable that we will return to a time when what are presently considered minor bacterial infections are fatal diseases.

Over-prescription and improper prescription habits by some physicians have caused an indiscriminate increase in the availability of antibiotics to the public. The patients are also partly responsible, since they will often improperly use the drug, thereby generating yet another population of bacteria that is resistant, in whole or in part, to traditional antibiotics.

The bacterial pathogens that have haunted humanity remain, in spite of the development of modem scientific practices to deal with the diseases that they cause. Drug resistant bacteria are now an increasing threat to the health of humanity. A new generation of antibiotics is needed to once again deal with the pending health threat that bacteria present.

Discovery of New Antibiotics

As more and more bacterial strains become resistant to the panel of available antibiotics, new antibiotics are required to treat infections. In the past, practitioners of pharmacology would have to rely upon traditional methods of drug discovery to generate novel, safe and efficacious compounds for the treatment of disease. Traditional drug discovery methods involve blindly testing potential drug candidate-molecules, often selected at random, in the hope that one might prove to be an effective treatment for some disease. The process is painstaking and laborious, with no guarantee of success. Today, the average cost to discover and develop a new drug exceeds US $500 million, an... d the average time from laboratory to patient is 15 years. Improving this process, even incrementally, would represent a huge advance in the generation of novel antimicrobial agents.

Newly emerging practices in drug discovery utilize a number of biochemical techniques to provide for directed approaches to creating new drugs, rather than discovering them at random. For example, gene sequences and proteins encoded thereby that are required for the proliferation of a cell or microorganism

make excellent targets since exposure of bacteria to compounds active against these targets would result in the inactivation of the cell or microorganism. Once a target is identified, biochemical analysis of that target can be used to discover or to design molecules that interact with and alter the functions of the target. Use of physical and computational techniques to analyze structural and biochemical properties of targets in order to derive compounds that interact with such targets is called rational drug design and offers great potential. Thus, emerging drug discovery practices use molecular modeling techniques, combinatorial chemistry approaches, and other means to produce and screen and/or design large numbers of candidate compounds.

Nevertheless, while this approach to drug discovery is clearly the way of the future, problems remain. For example, the initial step of identifying molecular targets for investigation can be an extremely time consuming task. It may also be difficult to design molecules that interact with the target by using computer modeling techniques. Furthermore, in cases where the function of the target is not known or is poorly understood, it may be difficult to design assays to detect molecules that interact with and alter the functions of the target. To improve the rate of novel drug discovery and development, methods of identifying important molecular targets in pathogenic cells or microorganisms and methods for identifying molecules that interact with and alter the functions of such molecular targets are urgently required.

Escherichia coli represents an excellent model system to understand bacterial biochemistry and physiology. The estimated 4288 genes scattered along the 4.6 x 106 base pairs of the
Escherichia coli (E. coli) chromosome offer tremendous promise for the understanding of bacterial biochemical processes. In turn, this knowledge will assist in the development of new tools for the diagnosis and treatment of bacteria-caused human disease. The entire E. coli genome has been sequenced, and this body of information holds a tremendous potential for application to the discovery and development of new antibiotic compounds. Yet, in spite of this accomplishment, the general functions or roles of many of these genes are still unknown. For example, the total number of proliferation-required genes contained within the E. coli genome is unknown, but has been variously estimated at around 200 to 700 (Armstrong, K. A. and Fan, D. P. Essential Genes in the metB-malB Region of Escherichia coli K12,1975, J. Bacteriol. 126: 48-55).

Staplzylococcus aureus is a Gram positive microorganism which is the causative agent of many infectious diseases. Local infection by Staphylococcus aureus can cause abscesses on skin and cellulitis in subcutaneous tissues and can lead to toxin-related diseases such as toxic shock and scalded skin syndromes. Staplzylococcus aureus can cause serious systemic infections such as osteomyelitis, endocarditis, pneumonia, and septicemia. Staplaylococcus aureus is also a common cause of food poisoning, often arising from contact between prepared food and infected food industry workers. Antibiotic resistant strains of S'taphylococcus aureus have recently been identified, including those that are now resistant to all available antibiotics, thereby severely limiting the options of care available to physicians.

Pseudomonas aeruginosa is an important Gram negative opportunistic pathogen. It is the most common Gram negative found in nosocomial infections. P. aeruginosa is responsible for 16% of nosocomial pneumonia cases, 12% of hospital-acquired urinary tract infections, 8% of surgical wound infections, and 10% of bloodstream infections. Immunocompromised patients, such as neutropenic cancer and bone marrow transplant patients, are particular susceptible to opportunistic infections. In this group of patients, P. aeruginosa is responsible for pneumonia and septicemia with attributable deaths reaching 30%. P. aeruginosa is also one of the most common and lethal pathogens responsible for ventilator-associated pneumonia in intubated patients, with directly attributable death rates reaching 38%. Although P. aeruginosa outbreaks in burn patients are rare, it is associated with 60% death rates. In the AIDS population, P. aeruginosa is associated with 50% of deaths. Cystic fibrosis patients are characteristically susceptible to chronic infection by P. aeruginosa, which is responsible for high rates of illness and death. Current antibiotics work poorly for CF infections (Van Delden & Igelwski. 1998. Emerging Infectious Diseases 4: 551-560; references therein).

The gram negative enteric bacterial genus, Salmonella, encompasses at least 2 species. One of these, S. enterica, is divided into multiple subspecies and thousands of serotypes or serovars (Brenner, et al. 2000 J. Clin. Microbiol. 38: 2465-2467). The S. enterica human pathogens include serovars Typhi, Paratyphi, Typhimurium, Cholerasuis, and many others deemed so closely related that they are variants of a widespread species. Worldwide, disease in humans caused by Saltizonella is a very serious problem. In many developing countries, S. enterica ser. Typhi still causes oftenfatal typhoid fever. This problem has been reduced or eliminated in wealthy industrial states.

However, enteritis induced by Salmonella is widespread and is the second most common disease caused by contaminated food in the United States (Edwards, BH 1999"Salmonella and Shigella species"Clin. Lab Med. 19 (3): 469-487). Though usually self-limiting in healthy individuals, others such as children, seniors, and those with compromising illnesses can be at much greater risk of serious illness and death.

Some S. enterica serovars (e. g. Typhimurium) cause a localized infection in the gastrointestinal tract. Other serovars (i. e. Typhi and Paratyphi) cause a much more serious systemic infection. In animal models, these roles can be reversed which has allowed the use of the relatively safe S. enterica ser. Typhimurium as a surrogate in mice for the typhoid fever agent, S. enterica ser.

Typhi. In mice, S. enterica ser Typhimurium causes a systemic infection similar in outcome to typhoid fever. Years of study of the Salrraonella have led to the identification of many determinants of virulence in animals and humans. Salmonella is interesting m its ability to localize to ana invade the intestinal epithelium, induce morphologic changes in target cells via injection of certain cellremodeling proteins, and to reside intracellularly in membrane-bound vesicles (Wallis, TS and
Galyov, EE 2000"Molecular basis of Salmonella-induced enteritis."Molec. Microb. 36: 997-1005;
Fallow, S"The evolution of pathogenicity in Escherichia, Shigella, and Salmonella,"Chap. 149 in
Neidhardt, et al. eds pp 2723-2729; Gulig, PA"Pathogenesis of Systemic Disease,"Chap. 152 in
Neidhardt, et al. ppp 2774-2787). The immediate infection often results in a severe watery diarrhea but Salmonella also can establish and maintain a subclinical carrier state in some individuals.

Spread is via food contaminated with sewage.

The gene products implicated in Sal7no7lella pathogenesis include type three secretion systems (TTSS), proteins affecting cytoplasmic structure of the target cells, many proteins carrying out functions necessary for survival and proliferation of Salmonella in the host, as well as "traditional"factors such as endotoxin and secreted exotoxins. Additionally, there must be factors mediating species-specific illnesses. Despite this most of the genomes of S. enterica ser. Typhi (see http ://www. sanger. ac. ulclProjects/S~typhi/for the genome database) and S. enterica ser.

Typhimurium (see http ://genome. wustl. edu/gsc/bacterial/salmonella. shtml for the genome database) are highly conserved and are mutually useful for gene identification in multiple serovars. The
Salmonella are a complex group of enteric bacteria causing disease similar to but distinct from other gram negative enterics such as E. coli and have been a focus of biomedical research for the last century.

Enterococcus faecalis, a Gram positive bacterium, is by far the most common member of the enterococci to cause infections in humans. Enterococcus faecium generally accounts for less than 20% of clinical isolates. Enterococci infections are mostly hospital-acquired though they are also associated with some community-acquired infections. Of nosocomial infections enterococci account for 12% of bacteremia, 15% of surgical wound infections, 14% of urinary tract infections, and 5 tol5% of endocarditis cases (Huycke, M. M., D. F., Sahm and M. S. Gilmore. 1998.

Emerging Infectious Diseases 4: 239-249). Additionally enterococci are frequently associated with intraabdominal and pelvic infections. Enterococci infections are often hard to treat because they are resistant to a vast array of antimicrobial drugs, including aminoglycosides, penicillin, ampicillin and vancomycin. The development of multiple-drug resistant (MDR) enterococci has made this bacteria a major concern for treating nosocomial infections.

Current drug discovery methods involve screening large number of prospective therapeutic compounds to identify those that are effective therapeutic agents or that can be optimized to provide an effective therapeutic agents. For example, the compounds to be evaluated for therapeutic activity may be members of a library of compounds generated by combinatorial chemistry or members of a library of natural products.

Unfortunately, current methods are laborious and time consuming and may yield compounds which have already been identified or which act on gene

products which are already targeted by an existing therapeutic agent. In addition, a large number of compounds have been identified which have antimicrobial activity but which cannot be administered to individuals suffering from infection due to the fact that their targets are unknown.

The above reasons underscore the urgency of developing new antibiotics that are effective against Escherichia coli, Stapllylococcus aureus, Enterococcus faecalis, Klebsiella pneufnoniae,
Pseudomonas aeruginosa, and Salmonella typhimurium. Accordingly, there is an urgent need for more novel methods to identify and characterize bacterial genomic sequences that encode gene products involved in proliferation, and are thereby potential new targets for antibiotic development.

Likewise, there is a need for rapid screening techniques which yield novel compounds or compounds which act on novel targets as well as a need for methods which permit the identification of the target on which a compound with antimicrobial activity acts.

Prior to the present invention, the discovery of Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pnemnoniae, Pseudorraonas aerugirzosa, and Salmonella typhimurium genes required for proliferation of the microorganism was a painstaking and slow process. Rapid screening techniques for identifying novel targets on which novel compounds act were undeveloped. While the detection and identification of new cellular drug targets within a
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, and Sal7izonella typhiiiiiuriui7i cell is key for novel antibiotic development and effective treatment, the current methods of drug target discovery available prior to this invention have required painstaking processes requiring years of effort.

Summary of the Invention
Some aspects of the present invention are described in the numbered paragraphs below.

1. A purified or isolated nucleic acid sequence comprising a nucleotide sequence consisting essentially of one of SEQ ID NOs: 1-6213, wherein expression of said nucleic acid inhibits proliferation of a cell.

2. The nucleic acid sequence of Paragraph 1, wherein said nucleotide sequence is complementary to at least a portion of a coding sequence of a gene whose expression is required for proliferation of a cell.

3. The nucleic acid of Paragraph 1, wherein said nucleic acid sequence is complementary to at least a portion of a nucleotide sequence of an RNA required for proliferation of a cell.

4. The nucleic acid of Paragraph 3, wherein said RNA is an RNA comprising a sequence of nucleotides encoding more than one gene product.

5. A purified or isolated nucleic acid comprising a fragment of one of SEQ ID NOs.: 1-6213, said fragment selected from the group consisting of fragments comprising at least 10, at least 20, at least 25, at least 30, at least 50 and more than 50 consecutive nucleotides of one of SEQ ID NOs : 1-6213.

6. The fragment of Paragraph 5, wherein said fragment is included in a nucleic acid obtained from an organism selected from the group consisting of Acijaetobacter baumannii,
Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candia parapsilosis, Candila guilliermondii, Candida krusei,
Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Clostridium perfringens, Coccidiodies immitis, Corynebacterium diptheriae,
Cryptococcus neofermans, Enterobacter cloacae, Enterococcus faecalis, Enterococuccus faecium,

Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis,
Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma penumonaie, Neisseria gonorrhoeae, Neisseria meningitidis,
Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii,
Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri,
Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptocous pyogenes, Treponema pallidum,
Ureaplasina urealyticuin, Vibrio cholerae, Vibrio parahaenzolyticus, Vibrio vuliiificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

7. The fragment of Paragraph 5, wherein said fragment is included in a nucleic acid obtained from an organism other than Escherichia coli.

8. A vector comprising a promoter operably linked to the nucleic acid of any one of
Paragraphs 1-7.

9. The vector of Paragraph 8, wherein said promoter is active in a microorganism selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroicles fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlzlaolderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium diff cile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumonaie, Neisseria gonorrhoeae, Neisseira meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

10. A host cell containing the vector of Paragraph 8 or Paragraph 9.

11. A purified or isolated antisense nucleic acid comprising a nucleotide sequence complementary to at least a portion of an intragenic sequence, intergenic sequence, sequences spanning at least a portion of two or more genes, 5'noncoding region, or 3'noncoding region within an operon comprising a proliferation-required gene whose activity or expression is inhibited by an antisense nucleic acid comprising the nucleotide sequence of one of SEQ ID NOs.: 1-6213.

12. The purified or isolated antisense nucleic acid of Paragraph 11, wherein said antisense nucleic acid is complementary to a nucleic acid from an organism

selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus funigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia penumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Crypotococcs neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pnemnoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella hae7nolytica, Pasteurella multocida, Pneumocystis carinfi, Proteus nlirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonelal enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyrogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia pestis and any species falling within the genera of any of the above species.

13. The purified or isolated antisense nucleic acid of Paragraph 11, wherein said nucleotide sequence is complementary to a nucleotide sequence of a nucleic acid from an organism other than E. coli.

14. The purified or isolated antisense nucleic acid of Paragraph 11, wherein said proliferation-required gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS. : 6214-42397.

15. A purified or isolated nucleic acid comprising a nucleotide sequence having at least 70% identity to a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, fragments comprising at least 25 consecutive nucleotides of SEQ ID NOs.: 1-6213, the nucleotide sequences complementary to SEQ ID NOs.: 1-6213 and the sequences complementary to fragments comprising at least 25 consecutive nucleotides of SEQ ID NOs.: 1-6213 as determined using
BLASTN version 2.0 with the default parameters.

16. The purified or isolated nucleic acid of Paragraph 15, wherein said nucleic acid is obtained from an organism selected from the group consisting of Acinetobacter baumannii
Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdoi feri, Bunlcholderia cepacia, Burlcholderia fuzgorurra, Burlcholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida Acrusei,
Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Clostridium perfringens, Coccidiodies immitis, Corynebacterium diptheriae,
Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium,
Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella penumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis,
Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis,

Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii,
Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudo7nonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri,
Shigella sonnei, Staphylococcus aureus, Stap1lylococcus epidermidis, Staphylococcus hae7nolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Strepotococcus pyrogens, Treponema pallidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersiraia pestis and any species falling within the genera of any of the above species.

17. The nucleic acid of Paragraph 15, wherein said nucleic acid is obtained from an organism other than E. coli.

18. A vector comprising a promoter operably linlced to a nucleic acid encoding a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of any one of SEQ ID NOs.: 1-6213.

19. The vector of Paragraph 18, wherein said nucleic acid encoding said polypeptide is obtained from an organism selected from the group consisting of Acinetobacter baunaannii,
Anaplasma marginale, Aspergillus Fac Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobactef jejuni, Caradida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermo71dii, Candida Airusei, Candida Acefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Clostridium perfiringens, Coccidiodies immitis, Corynebacterium diptheriae,
Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium,
Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplas7na capsulatum, Klebsiella pneumonaie, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis,
Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis,
Nocardia asterodies, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii,
Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri,
Shigella sonnei, Staphylococcus aureus, Staplzylococcus epidernidis, Staphylococcus haemolyticus,
Streptococcus pneu7noniae, Streptococcus mutans, Streptococcus pyogenes, Trepone7na pallidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

20. The vector of Paragraph 18, wherein said nucleotide sequence encoding said polypeptide is obtained from an organism other than E. coli.

21. A host cell containing the vector of Paragraph 18.

22. The vector of Paragraph 18, wherein said polypeptide comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4239878581.

23. The vector of Paragraph 18, wherein said promoter is operably linked to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 621442397.

24. A purified or isolated polypeptide comprising a polypeptide whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of any one of SEQ ID
NOs.: 1-6213, or a fragment selected from the group consisting of fragments comprising at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of one of the said polypeptides.

25. The polypeptide of Paragraph 24, wherein said polypeptide comprises an amino acid sequence of any one of SEQ ID NOs.: 42398-78581 or a fragment comprising at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

26. The polypeptide of Paragraph 24, wherein said polypeptide is obtained from an organism selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale,
Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdoiferi, Burkholderia cepacia, Burlcliolderia fungorum, Burlcholderia mallei, Caoapylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,
Candida parapsilosis, Candida guillierinondii, Candida krusei, Caizdida Icefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus irfluenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrlzalis, Mycobacteriurn avimn, Mycobacterium bovis,
Mycobacteriunz leprae, Mycobacterium tuberculosis, Mycoplasma getiitaliuiii, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneurnocystis carinii, Proteus rnirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

27. The polypeptide of Paragraph 24, wherein said polypeptide is obtained from an organism other than E. coli.

28. A purified or isolated polypeptide comprising a polypeptide having at least 25% amino acid identity to a polypeptide whose expression is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, or at least 25% amino acid identity to a fragment comprising at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of a polypeptide whose expression is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of
SEQ ID NOs.: 1-6213 as determined using FASTA version 3.0t78 with the default parameters.

29. The polypeptide of Paragraph 28, wherein said polypeptide has at least 25% identity to a polypeptide comprising one of SEQ ID NOs: 42398-78581 or at least 25% identity to a fragment comprising at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60 or more than 60 consecutive amino acids of a polypeptide comprising one of SEQ ID NOs.: 4239878581 as determined using FASTA version 3.0t78 with the default parameters.

30. The polypeptide of Paragraph 28, wherein said polypeptide is obtained from an organism selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale,

Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Buollaolderia cepacia, Burlcholderia fungoruna, Burlcholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia penumonaie, Chlamydia trachomatis, Clostridium acetobutylicum, CLostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella penumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacteriu7n leprae, Mycobacteriurn tuberculosis, Mycoplasma genitaliurn, Mycoplasma pneumonaie, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinfi, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella bodyii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcous aureus, Staphylococcus epider7nidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

31. The polypeptide of Paragraph 28, wherein said polypeptide is obtained from an organism other than E. coli.

32. An antibody capable of specifically binding the polypeptide of one of Paragraphs 28-31.

33. A method of producing a polypeptide, comprising introducing a vector comprising a promoter operably linked to a nucleic acid comprising a nucleotide sequence encoding a polypeptide whose expression is inhibited by an antisense nucleic acid comprising one of SEQ ID NOs.: 1-6213 into a cell.

34. The method of Paragraph 33, further comprising the step of isolating said polypeptide.

35. The method of Paragraph 33, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

36. The method of Paragraph 33, wherein said nucleic acid encoding said polypeptide is obtained from an organism selected from the group consisting of Acinetobacter baumannii, Anaplasma rnarginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliertnondii, Candida 1 usei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium actobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacte7ium diptheriae, Cfyptococcus neofornzans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumonaie, Neisseria gonorrhoeae, Neisseria meningitidis,

Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii,
Proteus mirabilis, Protells vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri,
Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogeizes, Treponenaa pallidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnifeans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

37. The method of Paragraph 33, wherein said nucleic acid encoding said polypeptide is obtained from an organism other than E. coli.

38. The method of Paragraph 33, wherein said promoter is operably linlced to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 621442397.

39. A method of inhibiting proliferation of a cell in an individual comprising inhibiting the activity or reducing the amount of a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213 or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product.

40. The method of Paragraph 39, wherein said method comprises inhibiting said activity or reducing said amount of a gene product in an organism selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida Icefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridum acetobutylicum,
Clostridium botulinum, Clostridum difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoforma7ls, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrlialis, Mycobacterium aviuln, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrlaoeae, Neisseria rneniyzgitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Praeurnocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella entrerica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticunz, Vibrio cholerae, Vibrio parahaerraolyticzts, Vibrio vulnifacans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

41. The method of Paragraph 39, wherein said method comprises inhibiting said activity or reducing said amount of a gene product in an organism other than E. coli.

42. The method of Paragraph 39, wherein said gene product is present in an organism other than E. coli.

43. The method of Paragraph 39, wherein said gene product comprises a polypeptide comprising a sequence selected from the group consisting of SEQ ID

NOs.: 42398-78581.

44. A method for identifying a compound which influences the activity of a gene product required for proliferation, said gene product comprising a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, said method comprising: contacting said gene product with a candidate compound; and determining whether said compound influences the activity of said gene product.

45. The method of Paragraph 44, wherein said gene product is from an organism selected from the group consisting of Acinetobacter bazcnaamaii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Canclida albicans, Caiadida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guiliermondii, Candida krusei, Candida kefry (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridum acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheride, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haerraophilus irfluenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneufnocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, PseudoYnonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulinificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

46. The method of Paragraph 44, wherein said gene product is from an organism other than E. coli.

47. The method of Paragraph 44, wherein said gene product is a polypeptide and said activity is an enzymatic activity.

48. The method of Paragraph 44, wherein said gene product is a polypeptide and said activity is a carbon compound catabolism activity.

49. The method of Paragraph 44, wherein said gene product is a polypeptide and said activity is a biosynthetic activity.

50. The method of Paragraph 44, wherein said gene product is a polypeptide and said activity is a transporter activity.

51. The method of Paragraph 44, wherein said gene product is a polypeptide and said activity is a transcriptional activity.

52. The method of Paragraph 44, wherein said gene product is a polypeptide and said activity is a DNA replication activity.

53. The method of Paragraph 44, wherein said gene product is a polypeptide and said activity is a cell division activity.

54. The method of Paragraph 44, wherein said gene product is an RNA.

55. The method of Paragraph 44, wherein said gene product is a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581.

56. A compound identified using the method of Paragraph 44.

57. A method for identifying a compound or nucleic acid having the ability to reduce the activity or level of a gene product required for proliferation, said gene product comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, said method comprising: (a) contacting a target gene or RNA encoding said gene product with a candidate compound or nucleic acid; and (b) measuring an activity of said target.

58. The method of Paragraph 57, wherein said target gene or RNA is from an organism selected from the group consisting of Acinetobacter bacumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Caridida guilliermondii, Candida Icrusei, Candida Icefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridum acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides irmnitis, Corynebacteriurn diptheriae, Cryptococcus neofornzans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinfi, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

59. The method of Paragraph 57, wherein said target gene or RNA is from an organism other than E. coli.

60. The method of Paragraph 57, wherein said gene product is from an organism other than E. coli.

61. The method of Paragraph 57, wherein said target is a messenger RNA molecule and said activity is translation of said messenger RNA.

62. The method of Paragraph 57, wherein said target is a messenger RNA molecule and said activity is transcription of a gene encoding said messenger RNA.

63. The method of Paragraph 57, wherein said target is a gene and said activity is transcription of said gene.

64. The method of Paragraph 57, wherein said target is a nontranslated RNA and said activity is processing or folding of said nontranslated RNA or assembly of said nontranslated RNA into a protein/RNA complex.

65. The method of Paragraph 57, wherein said target is a messenger RNA molecule encoding a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. : 42398-78581.

66. The method of Paragraph 57, wherein said target comprises a nucleic acid selected from the group consisting of SEQ ID NOS.: 6214-42397.

67. A compound or nucleic acid identified using the method of Paragraph 57.

68. A method for identifying a compound which reduces the activity or level of a gene product required for proliferation of a cell, wherein the activity or expression of said gene product is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, said method comprising the steps of : (a) providing a sublethal level of an antisense nucleic acid comprising a nucleotide sequence complementary to a nucleic acid comprising a nucleotide sequence encoding said gene product in a cell to reduce the activity or amount of said gene product in said cell, thereby producing a sensitized cell; (b) contacting said sensitized cell with a compound; and (c) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

69. The method of Paragraph 68, wherein said determining step comprises determining whether said compound inhibits the growth of said sensitized cell to a greater extent than said compound inhibits the growth of a nonsensitized cell.

70. The method of Paragraph 68, wherein said cell is a Gram positive bacterium.

71. The method of Paragraph 68, wherein said Gram positive bacterium is selected from the group consisting of Staphylococcus species, Streptococcus species, Enterococcus species,
Mycobacterium species, Clostridium species, and Bacillus species.

72. The method of Paragraph 68, wherein said bacterium is Stapltylococcus aureus.

73. The method of Paragraph 72, wherein said Staphylococcus species is coagulase negative.

74. The method of Paragraph 72, wherein said bacterium is selected from the group consisting of Staphylococcus aureus RN450 and Staphylococcus aureus RN4220.

75. The method of Paragraph 68, wherein said cell is an organism selected from the group consisting of Scnetobacter baunzannfi, Anaplas7na marginale, 4spergillus fumigatus,
Bacillus antllracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdojferi, Bur7cholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans,
Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis,
Candida guillier7nondii, Candida Acrusei, Candida Acefyr (also called Candida pseudotropicalis),
Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides irn7nitis, Corynebacterium diptheriae, Cryptococcus faeoformans, Eiaterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherchia coli, Haemophilius influenzae,
Helicobacter pylori, Histoplasnaa capsulatuna, Klebsiella pneunzoniae, Legionella pyaeunzophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitallium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella e7iterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,

Staphylococcus aureus, Staphylococcus epide77nidis, StaplZylococcus haemolyticus, Streptococcus p71eumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

76. The method of Paragraph 68, wherein said cell is not an E. coli cell.

77. The method of Paragraph 68, wherein said gene product is from an organism other than E. coli.

78. The method of Paragraph 68, wherein said antisense nucleic acid is transcribed from an inducible promoter.

79. The method of Paragraph 68, further comprising the step of contacting said cell with a concentration of inducer which induces transcription of said antisense nucleic acid to a sublethal level.

80. The method of Paragraph 68, wherein growth inhibition is measured by monitoring optical density of a culture growth solution.

81. The method of Paragraph 68, wherein said gene product is a polypeptide.

82. The method of Paragraph 81, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

83. The method of Paragraph 68, wherein said gene product is an RNA.

84. The method of Paragraph 68, wherein nucleic acid encoding said gene product comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

85. A compound identified using the method of Paragraph 68.

86. A method for inhibiting cellular proliferation comprising introducing an effective amount of a compound with activity against a gene whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 or a compound with activity against the product of said gene into a population of cells expressing said gene.

87. The method of Paragraph 86, wherein said compound is an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, or a proliferation-inhibiting portion thereof.

88. The method of Paragraph 86, wherein said proliferation inhibiting portion of one of SEQ ID NOs.: 1-6213 is a fragment comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 51 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

89. The method of Paragraph 86, wherein said population is a population of Gram positive bacteria.

90. The method of Paragraph 89, wherein said population of Gram positive bacteria is selected from the group consisting of a population of Staphylococcus species, Streptococcus species, Enterococcus species, Mycobacterium species, Clostridium species, and Bacillus species.

91. The method of Paragraph 86, wherein said population is a population of Staphylococcus aureus.

92. The method of Paragraph 91, wherein said population is a population of a bacterium selected from the group consisting of Staphylococcus aureus RN450 and Staphylococcus aureus RN4220.

93. The method of Paragraph 86, wherein said population is a population of a bacterium selected from the group consisting of Acinetobacter baunzannii, Anaplasma marginale,
Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,
Candida parapsilosis, Candida guilliermondii, Candida Acrusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridum acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Corynebacteriunz diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus infleunzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudoinoizas aeruginosa, Pseudoniotias putida, Pseudotizonas syriiigae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticmn, Vibrio cholenae, Vibrio parahaenzolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

94. The method of Paragraph 86, wherein said population is a population of an organism other than E. coli.

95. The method of Paragraph 86, wherein said product of said gene is from an organism other than E. coli.

96. The method of Paragraph 86, wherein said gene encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

97. The method of Paragraph 86, wherein said gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

98. A composition comprising an effective concentration of an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, or a proliferation-inhibiting portion thereof in a pharmaceutically acceptable carrier.

99. The composition of Paragraph 98, wherein said proliferation-inhibiting portion of one of SEQ ID NOs.: 1-6213 comprises at least 20, at least 25, at least 30, at least 50 or more than
50 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

100. A method for inhibiting the activity or expression of a gene in an operon required for proliferation wherein the activity or expression of at least one gene in said operon is inhibited by an antisense nucleic acid comprising a sequence selected from the group consisting of SEQ ID
NOs.: 1-6213, said method comprising contacting a cell in a cell population with an antisense nucleic acid complementary to at least a portion of said operon.

101. The method of Paragraph 100, wherein said antisense nucleic acid comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 or a proliferationinhibiting portion thereof.

102. The method of Paragraph 100, wherein said cell is selected from the group consisting of Acinetobacter baumannii, Anaplasma margiale, Aspergillus fumigatus, Bacills anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlcholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guiliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridum acetobutylicum,
Clostridium botulinzin2, Clostridiutn difficile, Clostridiuiii perfringeizs, Coccidioides iiii7nitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma gentitallium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomnonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Stap11ylococcus aureus, Stap1tylococcus epidernsidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia pestis and any species falling within the genera of any of the above species.

103. The method of Paragraph 100, wherein said cell is not an E. coli cell.

104. The method of Paragraph 100, wherein said gene is from an organism other than E. coli.

105. The method of Paragraph 100, wherein said cell is contacted with said antisense nucleic acid by introducing a plasmid which expresses said antisense nucleic acid into said cell population.

106. The method of Paragraph 100, wherein said cell is contacted with said antisense nucleic acid by introducing a phage which encodes said antisense nucleic acid into said cell population.

107. The method of Paragraph 100, wherein said cell is contacted with said antisense nucleic acid by expressing said antisense nucleic acid from the chromosome of cells in said cell population.

108. The method of Paragraph 100, wherein said cell is contacted with said antisense nucleic acid by introducing a promoter adjacent to a chromosomal copy of said antisense nucleic acid such that said promoter directs the transcription of said antisense nucleic acid.

109. The method of Paragraph 100, wherein said cell is contacted with said antisense nucleic acid by introducing a retron which expresses said antisense nucleic acid into said cell population.

110. The method of Paragraph 100, wherein said cell is contacted with said antisense nucleic acid by introducing a ribozyme into said cell-population, wherein a binding portion of said ribozyme comprises said antisense nucleic acid.

111. The method of Paragraph 100, wherein said cell is contacted with said antisense nucleic acid by introducing a liposome comprising said antisense nucleic acid into said cell.

112. The method of Paragraph 100, wherein said cell is contacted with said antisense nucleic acid by electroporation of said antisense nucleic acid into said cell.

113. The method of Paragraph 100, wherein said antisense nucleic acid is a fragment comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

114. The method of Paragraph 100 wherein said antisense nucleic acid is a synthetic oligonucleotide.

115. The method of Paragraph 100, wherein said gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

116. A method for identifying a gene which is required for proliferation of a cell comprising: (a) contacting a cell with an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, wherein said cell is a cell other than the organism from which said nucleic acid was obtained; (b) determining whether said nucleic acid inhibits proliferation of said cell; and (c) identifying the gene in said cell which encodes the mRNA which is complementary to said antisense nucleic acid or a portion thereof.

117. The method of Paragraph 116, wherein said cell is selected from the group consisting of Staphylococcus species, Streptococcus species, Enterococcus species, Mycobacterium species, Clostridium species, and Bacillus species.

118. The method of Paragraph 116 wherein said cell is selected from the group consisting of Acinetobacter baunlannfi, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Cadida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliernondii, Candida lc-usei, Candida Icefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridiufn perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrlzoeae, Neisseria meniragitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella fnultocida, Psaeumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Sahnonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhinaur iuna, Shigella boydii, Shigella dysenteriae, Shigella flexraeri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplsma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, yersinia enterocolitica, yersinia pestis and any species falling within the genera of any of the above species.

119. The method of Paragraph 116, wherein said cell is not E. coli.

120. The method of Paragraph 116, further comprising operably linking said antisense nucleic acid to a promoter which is functional in said cell, said promoter being included in a vector, and introducing said vector into said cell.

121. A method for identifying a compound having the ability to inhibit proliferation of a cell comprising: (a) identifying a homolog of a gene or gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 in a test cell, wherein said test cell is not the cell from which said nucleic acid was obtained; (b) identifying an inhibitory nucleic acid sequence which inhibits the activity of said homolog in said test cell; (c) contacting said test cell with a sublethal level of said inhibitory nucleic acid, thus sensitizing said cell; (d)

contacting the sensitized cell of step (c) with a compound; and (e) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not contain said inhibitory nucleic acid.

122. The method of Paragraph 121, wherein said determining step comprises determining whether said compound inhibits proliferation of said sensitized test cell to a greater extent than said compound inhibits proliferation of a nonsensitized test cell.

123. The method of Paragraph 121, wherein step (a) comprises identifying a nucleic acid homologous to a gene or gene product whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 or a nucleic acid encoding a homologous polypeptide to a polypeptide whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 by using an algorithm selected from the group consisting of BLASTN version 2.0 with the default parameters and FASTA version 3.0t78 algorithm with the default parameters to identify said homologous nucleic acid or said nucleic acid encoding a homologous polypeptide in a database.

124. The method of Paragraph 121 wherein said step (a) comprises identifying a homologous nucleic acid or a nucleic acid comprising a sequence of nucleotides encoding a homologous polypeptide by identifying nucleic acids which hybridize to said nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 or the complement of said nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213.

125. The method of Paragraph 121 wherein step (a) comprises expressing a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213 in said test cell.

126. The method of Paragraph 121, wherein step (a) comprises identifying a homologous nucleic acid or a nucleic acid encoding a homologous polypeptide in a test cell selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi,
BuyIzlaolderia cepacia, Burlclaolderia fungorunz, Burlclzoleeeria mallei, Cajrapylobacter jejuni,
Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida Acrusei, Ca71dida Acefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Hae7nophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitaliu77l, Mycoplasma pneumoniae, Neisseria gonorrlloeae, Neisseria fnerzingitidis, Nocardia astercides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

127. The method of Paragraph 121, wherein step (a) comprises identifying a homologous nucleic acid or a nucleic acid encoding a homologous polypeptide in a test cell other than E. coli.

128. The method of Paragraph 121, wherein said inhibitory nucleic acid is an antisense nucleic acid.

129. The method of Paragraph 121, wherein said inhibitory nucleic acid comprises an antisense nucleic acid to a portion of said homolog.

130. The method of Paragraph 121, wherein said inhibitory nucleic acid comprises an antisense nucleic acid to a portion of the operon encoding said homolog.

131. The method of Paragraph 121, wherein the step of contacting the cell with a sublethal level of said inhibitory nucleic acid comprises directly contacting the surface of said cell with said inhibitory nucleic acid.

132. The method of Paragraph 121, wherein the step of contacting the cell with a sublethal level of said inhibitory nucleic acid comprises transcribing an antisense nucleic acid complementary to at least a portion of the RNA transcribed from said homolog in said cell.

133. The method of Paragraph 121, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581.

134. The method of Paragraph 121, wherein said gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

135. A compound identified using the method of Paragraph 121.

136. A method of identifying a compound having the ability to inhibit proliferation comprising: (a) contacting a test cell with a sublethal level of a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 or a portion thereof which inhibits the proliferation of the cell from which said nucleic acid was obtained, thus sensitizing said test cell; (b) contacting the sensitized test cell of step (a) with a compound; and (c) determining the degree to which said compound inhibits proliferation of said sensitized test cell relative to a cell which does not contain said nucleic acid.

137. The method of Paragraph 136, wherein said determining step comprises determining whether said compound inhibits proliferation of said sensitized test cell to a greater extent than said compound inhibits proliferation of a nonsensitized test cell.

138. A compound identified using the method of Paragraph 136.

139. The method of Paragraph 136, wherein said test cell is selected from the group consisting of Acinetobacter baumanii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlholderia cepacza,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefry (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, C7yptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haenaophilus influesazae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterizi7iz leprae, Mycobacteriuni tubei-culosis, Mycoplasina genitaliuin, Mycoplasina pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium,m Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Steptococcus mutans, Steptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, yersinia enterocolitica.

Yersinia pestis and any species falling within the genera of any of the above species.

140. The method of Paragraph 136, wherein the test cell is not E. coli.

141. A method for identifying a compound having activity against a biological pathway required for proliferation comprising: (a) sensitizing a cell by providing a sublethal level of an antisense nucleic acid complementary to a nucleic acid encoding a gene product required for proliferation, wherein the activity or expression of said gene product is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID
NOs.: 1-6213, in said cell to reduce the activity or amount of said gene product; (b) contacting the sensitized cell with a compound; and (c) determining the degree to which said compound inhibits the growth of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

142. The method of Paragraph 141, wherein said determining step comprises determining whether said compound inhibits the growth of said sensitized cell to a greater extent than said compound inhibits the growth of a nonsensitized cell.

143. The method of Paragraph 141, wherein said cell is selected from the group consisting of bacterial cells, fungal cells, plant cells, and animal cells.

144. The method of Paragraph 141, wherein said cell is a Gram positive bacterium.

145. The method of Paragraph 144, wherein said Gram positive bacterium is selected from the group consisting of Staphylococcus species, Streptococcus species, Enterococcus species, Mycobacteriuna species, Clostridium species, and Bacillus species.

146. The method of Paragraph 145, wherein said Gram positive bacterium is Staplaylococcus aureus.

147. The method of Paragraph 146, wherein said Gram positive bacterium is selected from the group consisting of Staphylococcus aureus RN450 and Staphylococcus aureus RN4220.

148. The method of Paragraph 141, wherein said cell is selected from the group consisting of Acinetobacter baumannfi, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candias krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridiufn difficile, Clostridium perfringens, Coccidioides immitis,
Corpynebacterium diptheriae, C7yptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophyilus influenzae, Helicobacter pyrlori,
Histoplasma capsulatum, Klebsidella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumouiae, Neisseria gonorrlaoeae, Neisseria menifzgitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomo7las syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio Cholerae, Vibrio parahaeolyticus, Vibrio vulnificnas, Yersinia enterocolitica,
Yersinia pestis and any species falling with in the genera of any of the above species.

149. The method of Paragraph 141, wherein said cell is not an E. coli cell.

150. The method of Paragraph 141, wherein said gene product is from an organism other than E. coli.

151. The method of Paragraph 141, wherein said antisense nucleic acid is transcribed from an inducible promoter.

152. The method of Paragraph 141, further comprising contacting the cell with an agent which induces transcription of said antisense nucleic acid from said inducible promoter, wherein said antisense nucleic acid is transcribed at a sublethal level.

153. The method of Paragraph 141, wherein inhibition of proliferation is measured by monitoring the optical density of a liquid culture.

154. The method of Paragraph 141, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398
78581.

155. The method of Paragraph 141, wherein said nucleic acid encoding said gene product comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.:
6214-42397.

156. A compound identified using the method of Paragraph 141.

157. A method for identifying a compound having the ability to inhibit cellular proliferation comprising: (a) contacting a cell with an agent which reduces the activity or level of a gene product required for proliferation of said cell, wherein said gene product is a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213; (b) contacting said cell with a compound; and (c) determining whether said compound reduces proliferation of said contacted cell by acting on said gene product.

158. The method of Paragraph 157, wherein said determining step comprises determining whether said compound reduces proliferation of said contacted cell to a greater extent than said compound reduces proliferation of cells which have not been contacted with said agent.

159. The method of Paragraph 157, wherein said cell is selected from the group consisting of Acintobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burlcholderia fufzgoruna, Bur7cholderia rnallei, Carapylobacteo jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseuotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium aceobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faceium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneurraoniae, Legionella pnemnophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vaulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Samonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epiderinidis, Stapliylococcus haemolyticus, Streptococcus pneumoniae, Streptooccus mutans, Streptococcus

pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaenaolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

160. The method of Paragraph 157, wherein said cell is not an E. coli cell.

161. The method of Paragraph 157, wherein said gene product is from an organism other than E. coli,
162. The method of Paragraph 157, wherein said agent which reduces the activity or level of a gene product required for proliferation of said cell comprises an antisense nucleic acid to a gene or operon required for proliferation.

163. The method of Paragraph 157, wherein said agent which reduces the activity or level of a gene product required for proliferation of said cell comprises a compound known to inhibit growth or proliferation of a cell.

164. The method of Paragraph 157, wherein said cell contains a mutation which reduces the activity or level of said gene product required for proliferation of said cell.

165. The method of Paragraph 157, wherein said mutation is a temperature sensitive mutation.

166. The method of Paragraph 157, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581.

167. A compound identified using the method of Paragraph 157.

168. A method for identifying the biological pathway in which a proliferation-required gene or its gene product lies, wherein said gene or gene product comprises a gene or gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOs.: 1-6213, said method comprising: (a) providing a sublethal level of an antisense nucleic acid which inhibits the activity of said proliferation-required gene or gene product in a test cell; (b) contacting said test cell with a compound known to inhibit growth or proliferation of a cell, wherein the biological pathway on which said compound acts is known; and (c) determining the degree to which said proliferation of said test cell is inhibited relative to a cell which was not contacted with said compound.

169. The method of Paragraph 168, wherein said determining step comprises determining whether said test cell has a substantially greater sensitivity to said compound than a cell which does not express said sublethal level of said antisense nucleic acid.

170. The method of Paragraph 168, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581.

171. The method of Paragraph 168, wherein said test cell is selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumiatus, Bacillus anthracis, Bacteroides fi agilis, Bordetella pertussis, Borrelia burgdorferi, BurIcIzolderia cepacia, burkholderia fungorum, burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida Acrusei, Candida Acefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trahcomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherchia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,

Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, pasteuella multocida, Pneumocytis carinii, Proteus mirabilis, Proteus veulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Slaigella sonnei,
Staphylococcus aureus, Staplaylococcus epiderrnidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidium, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

* 172. The method of Paragraph 168, wherein said test cell is not an E. coli cell.

173. The method of Paragraph 168, wherein said gene product is from an organism other than E. coli.

174. A method for determining the biological pathway on which a test compound acts comprising: (a) providing a sublethal level of an antisense nucleic acid complementary to a proliferation-required nucleic acid in a first cell, wherein the activity or expression of said proliferation-required nucleic acid is inhibited by an antisense nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOs.: 1-6213 and wherein the biological pathway in which said proliferation-required nucleic acid or a protein encoded by said proliferation-required nucleic acid lies is known, (b) contacting said first cell with said test compound; and (c) determining the degree to which said test compound inhibits proliferation of said first cell relative to a cell which does not contain said antisense nucleic acid.

175. The method of Paragraph 174, wherein said determining step comprises determining whether said first cell has a substantially greater sensitivity to said test compound than a cell which does not express said sublethal level of said antisense nucleic acid.

176. The method of Paragraph 174, further comprising: (d) providing a sublethal level of a second antisense nucleic acid complementary to a second proliferation-required nucleic acid in a second cell, wherein said second proliferation-required nucleic acid is in a different biological pathway than said proliferation-required nucleic acid in step (a); and (e) determining whether said second cell does not have a substantially greater sensitivity to said test compound than a cell which does not express said sublethal level of said second antisense nucleic acid, wherein said test compound is specific for the biological pathway against which the antisense nucleic acid of step (a) acts if said first cell has a substantially greater sensitivity to said test compound than said second cell.

177. The method of Paragraph 174, wherein said first cell is selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Bunlcholderia cepacia,
Burltholderia fuagoruna, Burlcholderia naallei, Canzpylobacter jejuni, Cajzdida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida k7usei, Candida Acefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostrdium acetobutylicum,
Clostridium botulinun, clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Co7ynebacteriuna diptheriae, Cryptococcus neofornaans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigealla boydii, Shigella dysenteriae, Shigella glexneri, Shigella sonnei,

Staphylococcus aureus, Staplzylococcus epidernaidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

178. The method of Paragraph 174, wherein said first cell is not an E. coli cell.

179. The method of Paragraph 174, wherein said proliferation-required nucleic acid is from an organism other than E. coli.

180. A purified or isolated nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOs.: 1-6213.

181. A compound which interacts with a gene or gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of one of
SEQ ID NOs.: 1-6213 to inhibit proliferation.

182. The compound of Paragraph 181, wherein said gene product is a polypeptide comprising one of SEQ ID NOs.: 42398-78581.

183. The compound of Paragraph 181, wherein said gene comprises a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397.

184. A compound which interacts with a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence of one of SEQ ID NOs.: 1-6213 to inhibit proliferation.

185. A method for manufacturing an antibiotic comprising the steps of : screening one or more candidate compounds to identify a compound that reduces the activity or level of a gene product required for proliferation, said gene product comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213; and manufacturing the compound so identified.

186. The method of Paragraph 185, wherein said screening step comprises performing any one of the methods of Paragraphs 44,68,121,136,141, and 157.

187. The method of Paragraph 185, wherein said gene product is a polypeptide comprising one of SEQ ID NOs : 42398-78581.

188. A method for inhibiting proliferation of a cell in a subject comprising administering an effective amount of a compound that reduces the activity or level of a gene product required for proliferation of said cell, said gene product comprising a gene product whose activity or expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 to said subject.

189. The method of Paragraph 188 wherein said subject is selected from the group consisting of vertebrates, mammals, avians, and human beings.

190. The method of Paragraph 188, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581.

191. The method of Paragraph 188, wherein said cell is selected from the group consisting of Acinetobacter baumanni, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlclaolderia cepacia,
Burkholderia fungorum, burkholderia mallei, Camplylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliernlondii, Candida Acrusei, Candida Acefyr (also called Candida psuedotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perforingens, Coccidioides immitis, Corynebacterium diptheriae, C7yptococcus neoformans,

Enterobacter cloacae, Enterococcus faecalis, Enterococcus faeium, escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis,
Mycobacterium leprae, mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis,
Nocardia asteroides, Pasteurella haemolytica, Pasteurella mziltocida, Pneumocystis carinii, Proteus nairabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syrinage, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhirram ium, Slaigella boydii, Shigella dysenteriae,
Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus
pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia pestis and any species falling within the genera of any of the above species.

192. The method of Paragraph 188, wherein said cell is not E. coli.

193. The method of Paragraph 188, wherein said gene product is from an organism other than E. coli.

194. A purified or isolated nucleic acid consisting essentially of the coding sequence of one of SEQ ID NOs: 6214-42397.

195. A fragment of the nucleic acid of Paragraph 8, said fragment comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of
SEQ ID NOs: 6214-42397.

196. A purified or isolated nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of SEQ ID
NOs.: 6214-42397, fragments comprising at least 25 consecutive nucleotides of SEQ ID NOs.: 6214-42397, the nucleotide sequences complementary to SEQ ID NOs. : 6214-42397, and the nucleotide sequences complementary to fragments comprising at least 25 consecutive nucleotides of SEQ ID NOs.: 6214-42397 as determined using BLASTN version 2.0 with the default parameters.

197. The nucleic acid of Paragraph 196, wherein said nucleic acid is from an organism selected from the group consisting of Acinetobacter baumannii,
Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, borrelia burgdorferi,
Burkholderia cepacia, burkholderia fungorum, bukholderia mallei, Campylobacter jejuni,
Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei,
Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinuni, Clostridium di
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium,
Escherichia coli, Haemophilus influenzae,
Helicobacter pyrlori, Histoplamsa capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium aviurn, MycobacteriufZ bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitaliu7n, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis,
Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudonaoszas aeruginosa, Pseudomonas putida, Pseudornonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salrnoraella paratyplzi, Salmonella typhi, Salmonella typhinaur-ium, Slzigella boydii, Slaigella dyseriteriae,
Shigella flexneri, Slaigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptotoccus

pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, vibrio vulnifcans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

198. The nucleic acid of Paragraph 196, wherein said nucleic acid is from an organism other than E. coli.

199. A method of inhibiting proliferation of a cell comprising inhibiting the activity or reducing the amount of a gene product in said cell or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product in said cell, wherein said gene product is selected from the group consisting of a gene product having having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID
NOs : 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213.

200. The method of Paragraph 199, wherein said method comprises inhibiting said activity or reducing said amount of said gene product or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product in an organism selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus antlzracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdosferi, Burltholdenia cepacia, Burkholderia fungorum, burkholderia mallei, Campylobacte jejuni, Candida albicans, Candida glabrata (also called Torulposis glabrata), Candida tropicalis, Candida parapsilosis, Candida. guilliernaondii, Caracdida lusei, Candida Icefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfingens, Coccidioides immitis,
Coryebacterium diptheriae, Cryptococcus neformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, escherichia coli, Haemophilus influrnzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrlloeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocytis carinii, Proteurs mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas Putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulzificans, Yersinia erater-ocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

201. The method of Paragraph 199, wherein said method comprises inhibiting said activity or reducing said amount of said gene product or inhibiting the activity or reducing the amount of a nucleic acid encoding said gene product in an organism other than E. coli.

202. The method of Paragraph 199, wherein said gene product is from an organism other than E. coli.

203. The method of Paragraph 199, wherein said gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42398-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42398-78581.

204. The method of Paragraph 199, wherein said gene product is encoded by a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 621442397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucloetide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

205. A method for identifying a compound which influences the activity of a gene product required for proliferation comprising : contacting a candidate compound with a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-
6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213; and determining whether said candidate compound influences the activity of said gene product.

206. The method of Paragraph 205, wherein said gene product is from an organism selected from the group consisting of Acinetobacter baumannfi, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans,Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplassta capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes,Moraxella catarrhalis, Mycobacterium avium,mYcobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteusmirabilis, proteus vulgaris, Pseudomonas aeruginosa, pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staplzylococcus aureus, Staphylococcus epideriiiidis, Staplzylococcus haentolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaetnolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

207. The method of Paragraph 205, wherein said gene product is from an organism other than E. coli.

208. The method of Paragraph 205, wherein said gene product is a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42398-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42398-78581.

209. The method of Paragraph 205, wherein said gene product is encoded by a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 621442397, a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

210. A compound identified using the method of Paragraph 205.

211. A method for identifying a compound or nucleic acid having the ability to reduce the activity or level of a gene product required for proliferation comprising: (a) providing a target that is a gene or RNA, wherein said target comprises a nucleic acid that encodes a gene product selected from the group consisting of a gene product having having at least 70% nucleotide sequence identity as determined using
BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleic acid identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3. 0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID
NOs: 1-6213; (b) contacting said target with a candidate compound or nucleic acid; and (c) measuring an activity of said target.

212. The method of Paragraph 211, wherein said target gene or RNA is from an organism selected from the group consisting of Acinetobacter bautizaiinii, Anaplasfraa naargiraale,
Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Bor7elia
burgdorfe7-i, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,
Candida parapsilosis, Candida guillie77nondii, Candida Acrusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneunzoniae, Chlamydia tracho7natis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mcobacteri7n bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella mutocida, Pneumocystis carinii, Proteus mirabilis, proteus vulgaris,

Pseudomonas aeruginosa, pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhifnuriurfz, Slzigella boydii, Sliigella dyseiiteriae, Shigella flexneri, Slligella sonnei,
Staphylococcus aureus, Staphylococcus epidernaidis, Staphylococcus Tzae7nolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptotoccus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

213. The method of Paragraph 211, wherein said target gene or RNA is from an organism other than E. coli.

214. The method of Paragraph 211, wherein said gene product is from an organism other than E. coli.

215. The method of Paragraph 211, wherein said target is a messenger RNA molecule and said activity is translation of said messenger RNA.

216. The method of Paragraph 211, wherein said compound is a nucleic acid and said activity is translation of said gene product.

217. The method of Paragraph 211, wherein said target is a gene and said activity is transcription of said gene.

218. The method of Paragraph 211, wherein said target is a nontranslated RNA and said activity is processing or folding of said nontranslated RNA or assembly of said nontranslated RNA into a protein/RNA complex.

219. The method of Paragraph 211, wherein said target gene is a messenger RNA molecule encoding a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42398-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs : 42398-78581.

220. The method of Paragraph 11, wherein said target gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

221. A compound or nucleic acid identified using the method of Paragraph 211.

222. A method for identifying a compound which reduces the activity or level of a gene product required for proliferation of a cell comprising: (a) providing a sublethal level of an antisense nucleic acid complementary to a nucleic acid encoding said gene product in a cell to reduce the activity or amount of said gene product in said cell, thereby producing a sensitized cell, wherein said gene product is selected from the group consisting of a gene product having having at least 70% nucleic acid identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs : 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be

complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213; (b) contacting said sensitized cell with a compound; and (c) determining the degree to which said compound inhibits the growth of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

223. The method of Paragraph 222, wherein said determining step comprises determining whether said compound inhibits the growth of said sensitized cell to a greater extent than said compound inhibits the growth of a nonsensitized cell.

224. The method of Paragraph 222, wherein said sensitized cell is a Gram positive bacterium.

225. The method of Paragraph 224, wherein said Gram positive bacterium is selected from the group consisting of Staphylococcus species, Streptococcus species, Enterococcus species, Mycobacterium species, Clostridium species, and Bacillus species.

226. The method of Paragraph 225, wherein said bacterium is Staphylococcus aureus.

227. The method of Paragraph 224, wherein said Staphylococcus species is coagulase negative.

228. The method of Paragraph 226, wherein said bacterium is selected from the group consisting of Staphylococcus aureus RN450 and Staphylococcus aureus RN4220.

229. The method of Paragraph 222, wherein said sensitized cell is an organism selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus futtiigatus, Bacillus anthi-acis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdoiferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliemnondii, Candida Icrusei, Candida I efyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Co7ynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae,Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium,mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, pasteurella haemolytica, Pasteurella multocida, Pneumocystiscarinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Sahnonella cholerasuis, Salinonella etitei-ica, Saltizonella paratyphi, Salnzonella typhi, Salnionella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

230. The method of Paragraph 222, wherein said cell is an organism other than E. coli.

231. The method of Paragraph 222, wherein said gene product is from an organism other than E. coli.

232. The method of Paragraph 222, wherein said antisense nucleic acid is transcribed from an inducible promoter.

233. The method of Paragraph 222, further comprising the step of contacting said cell with a concentration of inducer which induces transcription of said

antisense nucleic acid to a sublethal level.

234. The method of Paragraph 222, wherein growth inhibition is measured by monitoring optical density of a culture medium.

235. The method of Paragraph 222, wherein said gene product is a polypeptide.

236. The method of Paragraph 235, wherein said polypeptide comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of
SEQ ID NOs.: 42398-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs : 42398-78581.

237. The method of Paragraph 222, wherein said gene product is an RNA.

238. The method of Paragraph 222, wherein said nucleic acid encoding said gene product comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleic acid identity as determined using BLASTN version 2.0 with the default parameters to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid which hybridizes to a sequence selected from the group consisting of
SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

239. A compound identified using the method of Paragraph 222.

240. A method for inhibiting cellular proliferation comprising introducing a compound with activity against a gene product or a compound with activity against a gene encoding said gene product into a population of cells expressing said gene product, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of
SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using
FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of
SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 16213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of
SEQ ID NOs : 1-6213.

241. The method of Paragraph 240, wherein said compound is an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, or a proliferation-inhibiting portion thereof.

242. The method of Paragraph 240, wherein said proliferation inhibiting portion of one of SEQ ID NOs.: 1-6213 is a fragment comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 51 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

243. The method of Paragraph 240, wherein said population is a population of Gram positive bacteria.

244. The method of Paragraph 243, wherein said population of Gram positive bacteria is selected from the group consisting of a population of Staphylococcus species, Streptococcus species, Enterococcus species, Mycobacterium species, Clostridium species, and Bacillus species.

245. The method of Paragraph 243, wherein said population is a population of Stapllylococcus aureus.

246. The method of Paragraph 245, wherein said population is a population of a bacterium selected from the group consisting of Staphylococcus aureus RN450 and Staphylococcus aureus RN4220.

247. The method of Paragraph 240, wherein said population is a population of a bacterium selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale,
Aspergillus fuTnigatus, Bacillus antlzracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderiamallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,
Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridiuin botuli7zuiii, Clostridiuiii difficile, Clostridiuni peifriizgeiis,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae,Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, pasteurella haemolytica, pasteurella mutocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudoiitottas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia pestis and any species falling within the genera of any of the above species.

248. The method of Paragraph 240, wherein said population is a population of an organism other than E. coli.

249. The method of Paragraph 240, wherein said product of said gene is from an organism other than E. coli.

250. The method of Paragraph 240, wherein said gene product is selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using
FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 4239878581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs : 42398-78581.

251. The method of Paragraph 240, wherein said gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

252. A preparation comprising an effective concentration of an antisense nucleic acid in a pharmaceutically acceptable carrier wherein said antisense nucleic

acid is selected from the group consisting of a nucleic acid comprising a sequence having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 1-6213 or a proliferation-inhibiting portion thereof, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions.

253. The preparation of Paragraph 252, wherein said proliferation-inhibiting portion of one of SEQ ID NOs.: 1-6213 comprises at least 10, at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

254. A method for inhibiting the activity or expression of a gene in an operon which encodes a gene product required for proliferation comprising contacting a cell in a cell population with an antisense nucleic acid comprising at least a proliferation-inhibiting portion of said operon in an antisense orientation, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs : 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213.

255. The method of Paragraph 254, wherein said antisense nucleic acid comprises a nucleotide sequence having at least 70% nucleotide sequence identity as determined using
BLASTN version 2.0 with the default parameters to a nucleotide seqence selected from the group consisting of SEQ ID NOs.: 1-6213, a proliferation inhibiting portion thereof, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid which comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ
ID NOs.: 1-6213 under moderate conditions.

256. The method of Paragraph 254, wherein said cell is selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia tracho7natis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haerraolytica, Pasteurella multocida, Pneumocystis carinii, Proteus nzioabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,

Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

257. The method of Paragraph 254, wherein said cell is not an E. coli cell.

258. The method of Paragraph 254, wherein said gene is from an organism other than E. coli.

259. The method of Paragraph 254, wherein said cell is contacted with said antisense nucleic acid by introducing a plasmid which transcribes said antisense nucleic acid into said cell population.

260. The method of Paragraph 254, wherein said cell is contacted with said antisense nucleic acid by introducing a phage which transcribes said antisense nucleic acid into said cell population.

261. The method of Paragraph 254, wherein said cell is contacted with said antisense nucleic acid by transcribing said antisense nucleic acid from the chromosome of cells in said cell population.

262. The method of Paragraph 254, wherein said cell is contacted with said antisense nucleic acid by introducing a promoter adjacent to a chromosomal copy of said antisense nucleic acid such that said promoter directs the synthesis of said antisense nucleic acid.

263. The method of Paragraph 254, wherein said cell is contacted with said antisense nucleic acid by introducing a retron which expresses said antisense nucleic acid into said cell population.

264. The method of Paragraph 254, wherein said cell is contacted with said antisense nucleic acid by introducing a ribozyme into said cell-population, wherein a binding portion of said ribozyme is complementary to said antisense oligonucleotide.

265. The method of Paragraph 254, wherein said cell is contacted with said antisense nucleic acid by introducing a liposome comprising said antisense oligonucleotide into said cell.

266. The method of Paragraph 254, wherein said cell is contacted with said antisense nucleic acid by electroporation of said antisense nucleic acid into said cell.

267. The method of Paragraph 254, wherein said antisense nucleic acid has at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence comprising at least 10, at least 20, at least 25, at least 30, at least 50 or more than 50 consecutive nucleotides of one of SEQ ID NOs.: 1-6213.

268. The method of Paragraph 254 wherein said antisense nucleic acid is a synthetic oligonucleotide.

269. The method of Paragraph 254, wherein said gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

270. A method for identifying a gene which is required for proliferation of a cell comprising: (a) contacting a cell with an antisense nucleic acid selected from the group consisting of a nucleic acid at least 70% nucleotide sequence identity as determined using
BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 or a proliferation-inhibiting portion thereof, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, wherein said cell is a cell other than the organism from which said nucleic acid was obtained; (b) determining whether said nucleic acid inhibits proliferation of said cell; and (c) identifying the gene in said cell which encodes the mRNA which is complementary to said antisense nucleic acid or a portion thereof.

271. The method of Paragraph 270, wherein said cell is selected from the group consisting of Staphylococcus species, Streptococcus species, Enterococcus species, Mycobacterium species, Clostridium species, and Bacillus species.

272. The method of Paragraph 270 wherein said cell is selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anth7acis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderiacepacia,
Burkholderia fungorum, Burkholderiamallei, Campylobacterjejuni, Candidaalbicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candicla Irusei, Candida Izefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionelle pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacteriumleprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Stap7zylococcus aureus, Staplaylococcus epidermidis, Staphylococcus haernolyticus, Streptococcus piteutnoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia pestis and any species falling within the genera of any of the above species.

273. The method of Paragraph 270, wherein said cell is not E. coli.

274. The method of Paragraph 270, further comprising operably linking said antisense nucleic acid to a promoter which is functional in said cell, said promoter being included in a vector, and introducing said vector into said cell.

275. A method for identifying a compound having the ability to inhibit proliferation of a cell comprising: (a) identifying a homolog of a gene or gene product whose activity or level is inhibited by an antisense nucleic acid in a test cell, wherein said test cell is not the microorgaism from which the antisense nucleic acid was obtained, wherein said antisense nucleic acid is selected from the group consisting of a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1
6213, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions; (b) identifying an inhibitory nucleic acid sequence which inhibits the activity of said homolog in said test cell; (c)

contacting said test cell with a sublethal level of said inhibitory nucleic acid, thus sensitizing said cell; (d) contacting the sensitized cell of step (c) with a compound; and (e) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not express said inhibitory nucleic acid.

276. The method of Paragraph 275, wherein said determining step comprises determining whether said compound inhibits proliferation of said sensitized test cell to a greater extent than said compound inhibits proliferation of a nonsensitized test cell.

277. The method of Paragraph 275, wherein step (a) comprises identifying a homologous nucleic acid to a gene or gene product whose activity or level is inhibited by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID
NOs. 1-6213 or a nucleic acid encoding a homologous polypeptide to a polypeptide whose activity or level is inhibited by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 by using an algorithm selected from the group consisting of BLASTN version 2.0 with the default parameters and FASTA version 3.0t78 algorithm with the default parameters to identify said homologous nucleic acid or said nucleic acid encoding a homologous polypeptide in a database.

278. The method of Paragraph 275 wherein said step (a) comprises identifying a homologous nucleic acid or a nucleic acid encoding a homologous polypeptide by identifying nucleic acids comprising nucleotide sequences which hybridize to said nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 or the complement of the nucleotide sequence of said nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213.

279. The method of Paragraph 275 wherein step (a) comprises expressing a nucleic acid having at least 70% nucleic acid identity as determined using BLASTN version 2.0 with the default parameters to a sequence selected from the group consisting of SEQ ID NOs. 1-6213 in said test cell.

280. The method of Paragraph 275, wherein step (a) comprises identifying a homologous nucleic acid or a nucleic acid encoding a homologous polypeptide in an test cell selected from the group consisting of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fun2igatus, Bacillus anthfracis, Bacteroides fi agilis, Bordetella pertussis, Borrelia burgdorferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Clalanaydia pneunzoniae, Clalarnydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica,Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma
urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,

Yersinia pestis and any species falling within the genera of any of the above species.

281. The method of Paragraph 275, wherein step (a) comprises identifying a homologous nucleic acid or a nucleic acid encoding a homologous polypeptide in a test cell other than E. coli.

282. The method of Paragraph 275, wherein said inhibitory nucleic acid is an antisense nucleic acid.

283. The method of Paragraph 275, wherein said inhibitory nucleic acid comprises an antisense nucleic acid to a portion of said homolog.

284. The method of Paragraph 275, wherein said inhibitory nucleic acid comprises an antisense nucleic acid to a portion of the operon encoding said homolog.

285. The method of Paragraph 275, wherein the step of contacting the cell with a sublethal level of said inhibitory nucleic acid comprises directly contacting said cell with said inhibitory nucleic acid.

286. The method of Paragraph 275, wherein the step of contacting the cell with a sublethal level of said inhibitory nucleic acid comprises expressing an antisense nucleic acid to said homolog in said cell.

287. The method of Paragraph 275, wherein said gene product comprises a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581.

288. The method of Paragraph 275, wherein said gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

289. A compound identified using the method of Paragraph 275.

290. A method of identifying a compound having the ability to inhibit proliferation comprising: (a) sensitizing a test cell by contacting said test cell with a sublethal level of an antisense nucleic acid, wherein said antisense nucleic acid is selected from the group consisting of a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOs. 1-6213 or a portion thereof which inhibits the proliferation of the cell from which said nucleic acid was obtained, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditionst; (b) contacting the sensitized test cell of step (a) with a compound; and (c) determining the degree to which said compound inhibits proliferation of said sensitized test cell relative to a cell which does not contain said antisense nucleic acid.

291. The method of Paragraph 290, wherein said determining step comprises determining whether said compound inhibits proliferation of said sensitized test cell to a greater extent than said compound inhibits proliferation of a nonsensitized test cell.

292. A compound identified using the method of Paragraph 290.

293. The method of Paragraph 290, wherein said test cell is selected from the group consisting of Acinetobacter baumannfi, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdo7feri, BufA7zholderia cepacia, Burlzholdenia fungorufn, Burlcholderia mallei, Carnpylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida

tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium pef ingens, Coccidioides imrnitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasnza capsulatum, Klebsiella pnemnoniae, Legionella pneurnophila, Listef ia monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudonzonas putida, Pseudonzonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia pestis and any species falling within the genera of any of the above species.

294. The method of Paragraph 290, wherein the test cell is not E. coli.

295. A method for identifying a compound having activity against a biological pathway required for proliferation comprising: (a) sensitizing a cell by providing a sublethal level of an antisense nucleic acid complementary to a nucleic acid encoding a gene product required for proliferation, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs : 1
6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213; (b) contacting the sensitized cell with a compound ; and (c) determining the extent to which said compound inhibits the growth of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

296. The method of Paragraph 295, wherein said determining step comprises determining whether said compound inhibits the growth of said sensitized cell to a greater extent than said compound inhibits the growth of a nonsensitized cell.

297. The method of Paragraph 295, wherein said cell is selected from the group consisting of bacterial cells, fungal cells, plant cells, and animal cells.

298. The method of Paragraph 295, wherein said cell is a Gram positive bacterium.

299. The method of Paragraph 298, wherein said Gram positive bacterium is selected from the group consisting of Staplzylococcus species, Streptococcus species, Enterococcus species, Mycobacterium species, Clostridium species, and Bacillus species.

300. The method of Paragraph 299, wherein said Gram positive bacterium is Staphylococcus aureus.

301. The method of Paragraph 298, wherein said Gram positive bacterium is selected from the group consisting of Staphylococcus aureus RN450 and Staphylococcus aureus RN4220.

302. The method of Paragraph 295, wherein said cell is selected from the group consisting of Acinetobacter bauntannii, Anaplasnaa naargirzale, Aspergillus fmnigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guillierrnon. dii, Candida Iwusei, Candida Icefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria inonocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacteniunz tuberculosis, Mycoplasrraa genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimuriuni, Slaigella boydii, S7aigella dysenteriae, Slaigella flexneri, Slaigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio paralaaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

303. The method of Paragraph 295, wherein said cell is not an E. coli cell.

304. The method of Paragraph 295, wherein said gene product is from an organism other than E. coli.

305. The method of Paragraph 295, wherein said antisense nucleic acid is transcribed from an inducible promoter.

306. The method of Paragraph 305, further comprising contacting the cell with an agent which induces expression of said antisense nucleic acid from said inducible promoter, wherein said antisense nucleic acid is expressed at a sublethal level.

307. The method of Paragraph 295, wherein inhibition of proliferation is measured by monitoring the optical density of a liquid culture.

308. The method of Paragraph 295, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

309. The method of Paragraph 295, wherein said nucleic acid encoding said gene product comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

310. A compound identified using the method of Paragraph 295.

311. A method for identifying a compound having the ability to inhibit cellular proliferation comprising: (a) contacting a cell with an agent which reduces the activity or level of a gene product required for proliferation of said cell, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213; (b) contacting said cell with a compound; and (c) determining the degree to which said compound reduces proliferation of said contacted cell relative to a cell which was not contacted with said agent.

312. The method of Paragraph 311, wherein said determining step comprises determining whether said compound reduces proliferation of said contacted cell to a greater extent than said compound reduces proliferation of cells which have not been contacted with said agent.

313. The method of Paragraph 311, wherein said cell is selected from the group consisting of Acinetobacter b aumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burlcholderia mallei, Canapylobacter jejuni, Caadida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guillierinondii, Candida Ivusei, Candida Icejyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Co7ynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrTzalis, Mycobacteriufn aviuna, Mycobacterium bovis,
Mycobacteriun2 leprae, Mycobacteriurn tubejculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimuriu7n, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella so7mei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneu7noniae, Streptococcus mutans, St7eptococcus pyogenes, Trepo7zena pallidum, Ureaplasma urealyticunz, Vibrio cholerae, Vibrio parahae7nolyticus, Vibrio vulizificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

314. The method of Paragraph 311, wherein said cell is not an E. coli cell.

315. The method of Paragraph 311, wherein said gene product is from an organism other than E. coli.

316. The method of Paragraph 311, wherein said agent which reduces the activity or level of a gene product required for proliferation of said cell comprises

an antisense nucleic acid to a gene or operon required for proliferation.

317. The method of Paragraph 311, wherein said agent which reduces the activity or level of a gene product required for proliferation of said cell comprises a compound known to inhibit growth or proliferation of a cell.

318. The method of Paragraph 311, wherein said cell contains a mutation which reduces the activity or level of said gene product required for proliferation of said cell.

319. The method of Paragraph 311, wherein said mutation is a temperature sensitive mutation.

320. The method of Paragraph 311, wherein said gene product comprises a gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

321. A compound identified using the method of Paragraph 311.

322. A method for identifying the biological pathway in which a proliferation-required gene product or a gene encoding a proliferation-required gene product lies comprising: (a) providing a sublethal level of an antisense nucleic acid which inhibits the activity or reduces the level of said gene encoding a proliferation-required gene product or said said proliferation-required gene product in a test cell, wherein said proliferation required gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs : 1-
6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213; (b) contacting said test cell with a compound known to inhibit growth or proliferation of a cell, wherein the biological pathway on which said compound acts is known ; and (c) determining the degree to which said compound inhibits proliferation of said test cell relative to a cell which does not contain said antisense nucleic acid.

323. The method of Paragraph 322, wherein said determining step comprises determining whether said test cell has a substantially greater sensitivity to said compound than a cell which does not express said sublethal level of said antisense nucleic acid.

324. The method of Paragraph 322, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398
78581.

325. The method of Paragraph 322, wherein said test cell is selected from the group consisting of Acinetobacter baumannii, Anaplasma naarginale, Aspergillus furraigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlclzolderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliert7zoizdii, Candida krusei, Candida Icefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia

pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus iruenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacteriunz leprae, Mycobacteriuna tuberculosis, Mycoplasrraa genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinfi, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphyloccus epidermidis, Staphylococcus haemolyticus, Streptococcus pszeunzoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

326. The method of Paragraph 322, wherein said test cell is not an E. coli cell.

327. The method of Paragraph 322, wherein said gene product is from an organism other than E. coli.

328. A method for determining the biological pathway on which a test compound acts comprising : (a) providing a sublethal level of an antisense nucleic acid complementary to a proliferation-required nucleic acid in a cell, thereby producing a sensitized cell, wherein said antisense nucleic acid is selected from the group consisting of a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID
NOs: 1-6213 or a proliferation-inhibiting portion thereof, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions and wherein the biological pathway in which said proliferation-required nucleic acid or a protein encoded by said proliferation required polypeptide lies is known, (b) contacting said cell with said test compound; and (c) determining the degree to which said compound inhibits proliferation of said sensitized cell relative to a cell which does not contain said antisense nucleic acid.

329. The method of Paragraph 328, wherein said determining step comprises
determining whether said sensitized cell has a substantially greater sensitivity to said test compound than a cell which does not express said sublethal level of said antisense nucleic acid.

330. The method of Paragraph 328, further comprising: (d) providing a sublethal level of a second antisense nucleic acid complementary to a second proliferation-required nucleic acid in a second cell, wherein said second proliferation-required nucleic acid is in a different biological pathway than said proliferation-required nucleic acid in step (a); and (e) determining whether said second cell does not have a substantially greater sensitivity to said test compound than a cell which does not express said sublethal level of said second antisense nucleic acid, wherein said test compound is specific for the biological pathway against which the antisense nucleic acid of step (a) acts if said sensitized cell has substantially greater sensitivity to said test compound than said second cell.

331. The method of Paragraph 328, wherein said sensitized cell is selected from the group consisting of Acinetobacter baumannii, Anaplasnaa rraarginale, Aspergillus fumigatus,
Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei,

Campylobacter jejuni, Candida albicans,
Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis,
Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis),
Candida dubliniensis, Clalamydia pneumoniae, Clalanzydia trachomatis, Clostridiurn acetobutylicum, Clostridiuna botuliz7n, Clostridium difficile, Clostridimn perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatunz, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staplaylococcus epidenrnidis, Staphylococcus haenzolyticus, Streptococcus pneumoniae, Streptococcus mutans, Strepotococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

332. The method of Paragraph 328, wherein said sensitized cell is not an E. coli cell.

333. The method of Paragraph 328, wherein said proliferation-required nucleic acid is from an organism other than E. coli.

334. A compound which inhibits proliferation by interacting with a gene encoding a gene product required for proliferation or with a gene product required for proliferation, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using
BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213.

335. The compound of Paragraph 334, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a sequence selected from the group consisting of SEQ ID NOs.: 42398-78581.

336. The compound of Paragraph 334, wherein said gene comprises a nucleic acid selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate condtions.

337. A method for manufacturing an antibiotic comprising the steps of : screening one or more candidate compounds to identify a compound that reduces the activity or level of a gene product required for proliferation wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1
6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs : 1-6213; and manufacturing the compound so identified.

338. The method of Paragraph 337, wherein said screening step comprises performing any one of the methods of Paragraphs 205,211,222,275,290,295,311.

339. The method of Paragraph 337, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581.

340. A method for inhibiting proliferation of a cell in a subject comprising administering an effective amount of a compound that reduces the activity or level of a gene product required for proliferation of said cell, wherein said gene product is selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213.

341. The method of Paragraph 340 wherein said subject is selected from the group consisting of vertebrates, mammals, avians, and human beings.

342. The method of Paragraph 340, wherein said gene product comprises a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581.

343. The method of Paragraph 340, wherein said cell is selected from the group consisting of Acinetobacter baumannEi, Anaplasma marginale, Aspergillus funZigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdofferi, Burlzlaolderia cepacia,
BurAcholderia fungorum, Burlcholderia mallei, Ca7npylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida, kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,

Corynebacterium diptheriae, C7yptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasnia capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria fnonocytogenes, Moraxella catarrhalis, Mycobacterium aviuna, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria nneningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staplzylococcus auneus, Staphylococcus epidermidis, Staphylococcus haernolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

344. The method of Paragraph 340, wherein said cell is not E. coli.

345. The method of Paragraph 340, wherein said gene product is from an organism other than E. coli.

346. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

347. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtainin g a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 6214-42397 is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

348. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

349. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70%

nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:
1-6213 is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

* 350. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

351. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain in said culture overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938
78581 is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture.

352. The method of Paragraph 346,347,348,349,350 or 351, wherein said culture includes at least one strain which does not overexpresses a gene product which is essential for proliferation of said organism.

353. The method of Paragraph 346,347,348,349,350 or 351, wherein said strains which overexpress said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linlced to a regulatable promoter.

354. The method of Paragraph 346,347,348,349,350 or 351, wherein said strains which overexpress said gene products a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a constitutive promoter.

355. The method of Paragraph 346,347,348,349,350 or 351, wherein said identification step comprises determining the nucleotide sequence of a nucleic acid encoding said gene product in said cell which proliferated more rapidly in said culture.

356. The method of Paragraph 346,347,348,349,350 or 351, wherein said identification step comprises performing an amplification reaction to identify the nucleic acid encoding said gene product in said cell which proliferated more rapidly in said cell culture.

357. The method of Paragraph 356, wherein the products of said amplification reaction are labeled with a detectable dye.

358. The method of Paragraph 346,347,348,349,350 or 351, wherein said identification step comprises performing a hybridization procedure.

359. The method of Paragraph 346,347,348,349,350 or 351, wherein said identification step comprises contacting a nucleic acid array with a nucleic acid encoding said gene product in said cell which proliferated more rapidly in said cell culture.

360. The method of Paragraph 346,347,348,349,350 or 351, wherein said organism is selected from the group consisting of bacteria,. fungi, and protozoa.

361. The method of Paragraph 346,347,348,349,350 or 351, wherein said culture is a culture of an organism selected from the group consisting of Acinetobacter baumannii, Anaplasma naaf ginale, Aspergillus furnigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis,
Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata),
Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans,
Enterobacter cloacae, Enterococcus faecalis, Eratet-ococcus faeciutn, Escherichia coli,
Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoraiae,
Legionella pneumophila, listeria monocytogenes, Moraxella catarrhalis, Myclobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salrnofiella chOlerasuis, Salfnonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri,
Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

362. The method of Paragraph 346,347,348,349,350 or 351, wherein said compound is obtained from a library of natural compounds.

363. The method of Paragraph 346,347,348,349,350 or 351, wherein said compound is obtained from a library of synthetic compounds.

364. The method of Paragraph 346,347,348,349,350 or 351, wherein said compound is present in a crude or partially purified state.

365. The method of Paragraph 346, 347,348,349,350 or 351, further comprising determining whether said gene product in said strain which proliferated more

rapidly in said culture has a counterpart in at least one other organism.

366. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential. for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed; contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

367. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
6214-42397 is overexpressed; contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

368. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs. : 42938-78581 is overexpressed; contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

369. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1
6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed; contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more

rapidly on said solid medium.

370. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of
SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed; contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

371. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining an array of strains on a solid growth medium wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938
78581 is overexpressed; contacting said array of strains with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly on said solid medium.

372. The method of Paragraph 366,367,368,369,370 or 371, wherein at least one strain in said array does not overexpresses a gene product which is essential for proliferation of said organism.

373. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

374. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

375. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID

NOs.: 42938-78581 is overexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

376. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

377. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

378. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, wherein each culture comprises a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is overexpressed in a strain whose proliferation is inhibited by said compound.

379. The method of Paragraph 373,374,375,376,377 or 378, wherein at least one strain in said plurality of cultures does not overexpress a gene product which is essential for proliferation of said organism.

380. A method of profiling a compound's activity comprising: performing the method of Paragraph 346 on a first culture using a first compound; performing the method of Paragraph 346 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

381. A method of profiling a compound's activity comprising: performing the method of Paragraph 347 on a first culture using a first compound; performing the method of Paragraph 347 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

382. A method of profiling a compound's activity comprising: performing the method of Paragraph 348 on a first culture using a first compound; performing the method of Paragraph 348 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

383. A method of profiling a compound's activity comprising: performing the method of Paragraph 349 on a first culture using a first compound; performing the method of Paragraph 349 on a second culture using a second compound ; and comparing the strains identified in said first culture to the strains identified in said second culture.

384. A method of profiling a compound's activity comprising: performing the method of Paragraph 350 on a first culture using a first compound; performing the method of Paragraph 350 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

385. A method of profiling a compound's activity comprising : performing the method of Paragraph 351 on a first culture using a first compound; performing the method of Paragraph 351 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

386. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

387. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 6214-42397 is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

388. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism ; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

389. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited

by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:
1-6213 is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

390. A method of profiling a first compound's activity comprising : growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

391. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein each strain in said array overexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

392. The method of any one of Paragraphs 380,381,382,383,384,385,386,387,388, 389,390 or 391, wherein said first compound is present in a crude or partially purified state.

393. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

394. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein

said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts ; and identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

395. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising : obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

396. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:
1-6213 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

397. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

398. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938
78581 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress said gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture.

399. The method of Paragraph 393,394,395,396,397 or 398, wherein at least one strain in said culture does not underexpresses a gene product which is essential for proliferation of said organism.

400. The method of Paragraph 393,394,395,396,397 or 398, wherein said strains which underexpresess said gene products comprise a nucleic acid complementary to at least a portion of a gene encoding said gene product which is essential for proliferation of said organism operably linked to a regulatable promoter.

401. The method of Paragraph 393,394,395,396,397 or 398, wherein said strains which underexpress said gene products express an antisense nucleic acid complementary to at least a portion of a gene encoding said gene product which is essential for proliferation of said organism, wherein expression of said antisense nucleic acid reduces expression of said gene product in said strain.

402. The method of Paragraph 393,394,395,396,397 or 398, wherein said identification step comprises determining the nucleotide sequence of a nucleic acid encoding said gene product in said strain which proliferated more slowly.

403. The method of Paragraph 393,394,395,396,397 or 398, wherein said identification step comprises performing an amplification reaction to identify the nucleic acid encoding said gene product in said cell which proliferated more slowly.

404. The method of Paragraph 393,394,395,396,397 or 398, wherein the products of said amplification reaction are labeled with a detectable dye.

405. The method of Paragraph 404, wherein said identification step comprises performing a hybridization procedure.

406. The method of Paragraph 393,394,395,396,397 or 398, wherein said identification step comprises contacting a nucleic acid array with a nucleic acid encoding said gene product in said cell which proliferated more slowly.

407. The method of Paragraph 393,394,395,396,397 or 398, wherein said organism is selected from the group consisting of bacteria, fungi, protozoa.

408. The method of Paragraph 393,394,395,396,397 or 398, wherein said culture is a culture of an organism selected from the group consisting of Acinetobacter baunlannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis,
Borrelia burgdorferi, Burlcholderia cepacia, Burkholderia fungorum, Burkholderia mallei,
Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata),
Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans,
Enterobacter cloacae, Enterococcus faecalis, Enterococclls Faecium, Escherichia coli,

Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haenaolytica, Pasteurella rnultocida, Pneumocystis carizii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, S/ligella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Trepo71ema pallidum,
Ureaplasma urealyticuam, Vibrio cholerae, Tibrio parahaemolyticzcs, Tjibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

409. The method of Paragraph 393,394,395,396,397 or 398, wherein said compound is obtained from a library of natural compounds.

410. The method of Paragraph 393,394,395,396,397 or 398, wherein said compound is obtained from a library of synthetic compounds.

411. The method of Paragraph 393,394,395,396,397 or 398, wherein said compound is present in a crude or partially purified state.

412. The method of Paragraph 393,394,395,396,397 or 398, further comprising determining whether said gene product in said strain which proliferated more slowly in said culture has a counterpart in at least one other organism.

413. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

414. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed; contacting each of said cultures with a different concentration of said compound ; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

415. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

416. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, each culture. comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic

acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1 6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is underexpressed; contacting each of said cultures with a different concentration of said compound ; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

417. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising : obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

418. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures, each culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is underexpressed; contacting each of said cultures with a different concentration of said compound; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

419. A method of profiling a compound's activity comprising: performing the method of Paragraph 393 on a first culture using a first compound; performing the method of Paragraph 393 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

420. A method of profiling a compound's activity comprising: performing the method of Paragraph 394 on a first culture using a first compound; performing the method of Paragraph 394 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

421. A method of profiling a compound's activity comprising: performing the method of Paragraph 395 on a first culture using a first compound; performing the method of Paragraph 395 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

422. A method of profiling a compound's activity comprising performing the method of Paragraph 396 on a first culture using a first compound; performing the method of Paragraph 396 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

423. A method of profiling a compound's activity comprising performing the method of Paragraph 397 on a first culture using a first compound; performing the method of Paragraph 397 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

424. A method of profiling a compound's activity comprising performing the method of Paragraph 398 on a first culture using a first compound; performing the method of Paragraph 398 on a second culture using a second compound; and comparing the strains identified in said first culture to the strains identified in said second culture.

425. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

426. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

427. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

428. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 16213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and

comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

429. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

430. A method of profiling a first compound's activity comprising: growing an array of strains on a first solid medium comprising said first compound and on a second solid medium comprising a second compound, wherein said array comprises a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of an organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is underexpressed, and wherein said first compound and said second compound inhibit the proliferation of said organism; and comparing the pattern of strains which grow on said first solid medium with the pattern of strains which grow on said second solid medium.

431. The method of any one of Paragraphs 419,420,421,422,423,424,425,426,427, 428,429 or 430, wherein said first compound is present in a crude or partially purified state.

432. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed ; contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

433. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 6214-42397 is underexpressed ; contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

434. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed; contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of

expression of said gene products which are essential for proliferation of said organism; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

435. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs. : 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:
1-6213 is underexpressed; contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

436. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed; contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

437. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a plurality of cultures comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-
78581 is underexpressed; contacting each of said plurality of cultures with a varying concentration of a regulatory agent which regulates the level of expression of said gene products which are essential for proliferation of said organism; and identifying the gene product which is underexpressed in a strain whose rate of proliferation is reduced by said compound.

438. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed.

439. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed.

440. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed.

441. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed.

442. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 621442397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed.

443. A culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed.

444. The culture of Paragraph 438,439,440,441,442 or 443, wherein said strains which overexpresess said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a regulatable promoter.

445. The culture of Paragraph 438,439,440,441,442 or 443, wherein said strains which overexpresess said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linlced to a constitutive promoter.

446. The culture of Paragraph 438,439,440,441,442 or 443, wherein said culture is a culture of an organism selected from the group consisting of Acinetobacter baunaannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi,

Bm Icholderia cepacia, Burlcholderia furzgorum, Burlilzolderia nzallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata),
Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida 1cefyr (also called Candida pseudonopicalis), Candida dubliniensis, Clilatnydia pneumofriae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans,
Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli,
Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae,
Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudofnonas aeruginosa, Pseudomonas putida, Pseudorraonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri,
Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

447. A culture comprising a a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is underexpressed.

448. A culture comprising a a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed.

449. A culture comprising a a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed.

450. A culture comprising a a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is underexpressed.

451. A culture comprising a a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 621442397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed.

452. A culture comprising a a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is underexpressed.

453. The culture of Paragraph 447,448,449,450,451 or 452, wherein said strains which underexpress said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a regulatable promoter.

454. The culture of Paragraph 447,448,449,450,451 or 452, wherein said strains which underexpress said gene products comprise a nucleic acid encoding said gene product which is essential for proliferation of said organism operably linked to a constitutive promoter.

455. The culture of Paragraph 447,448,449,450,451 or 452, wherein said culture is a culture of an organism selected from the group consisting of Acinetobacter baumannii, Anaplas7na marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis,
Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata),
Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Co7ynebacte7ium diptheriae, Cryptococcus neoformans,
Enterobacter cloacae, Enterococcus faecalis, En. terococcus faeciuna, Escherichia coli,
Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacteriu7n bovis, Mycobacteriu77t leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,, Salmonella cholerasuis, Salnzonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhisnuriurra, Slaigella boydii, Shigella dysenteriae, Slzigella flexneri,
Shigella sonnei, Staphylococcus aureus, Stpahylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

456. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited

by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

457. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

458. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

459. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1
6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed ; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which

proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

460. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

461. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain overexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the overexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the overexpressed genes, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed ; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which do not overexpress said gene product on which said compound acts, such that strains which overexpress said gene product on which said compound acts proliferate more rapidly than strains which do not overexpress said gene product on which said compound acts; and identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

462. The method of Paragraph 456,457,458,459,460 or 461, wherein the nucleotide sequence of each of the genes encoding an overexpressed gene product has been altered by replacing the native promoters of said genes with promoters which facilitate overexpression of said gene products.

463. The method of Paragraph 456,457,458,459,460 or 461, wherein the nucleotide sequence of each of the genes encoding an overexpressed gene product has been altered by inserting a regulatory element into the native promoters of said genes with a promoter which facilitates overexpression of said gene products.

464. The method of Paragraph 463, wherein said regulatory element is selected from the group consisting of a regulatable promoter, an operator which is recognized by a repressor, a nucleotide sequence which is recognized by a transcriptional activator, a transcriptional terminator, a nucleotide sequence which introduces a bend in the DNA and an upstream activating sequence.

465. The method of Paragraph 456,457,458,459,460 or 461, wherein the step of identifying the gene product which is overexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene comprises performing an amplification reaction and detecting a unique amplification product corresponding to said gene.

466. The method of Paragraph 462, wherein the native promoter of each of the genes encoding a gene product essential for proliferation is replaced with the

same promoter.

467. The method of Paragraph 462, wherein the native promoters of the genes encoding gene products essential for proliferation are replaced with a plurality of promoters selected to give a desired expression level for each gene product.

468. The method of Paragraph 462, wherein said promoters which replaced the native promoters in each strain comprise regulatable promoters.

469. The method of Paragraph 462, wherein said promoters which replaced the native promoters in each strain each strain comprise constitutive promoters.

470. The method of Paragraph 456, 457, 458, 459, 460 or 461, wherein said organism is selected from the group consisting of bacteria, fungi, and protozoa.

471. The method of Paragraph 456,457,458,459,460 or 461, wherein said culture is a culture of an organism selected from the group consisting of Acinetobacter baumannii, Anaplasma ma7gi7lale, Aspergillus fun ? igatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlcholderia cepacia, Burlcholderia fungorum, Burlcholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guillierrraondii, Candida Icrusei, Candida 7cefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfiingens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae,. Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

472. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the underexpressed genes and wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1 6213 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

473. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique

product corresponding to each of the underexpressed genes and wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

474. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the underexpressed genes, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is underexpressed ; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

475. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the underexpressed genes, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-
6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

476. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the underexpressed genes, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.:

6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

477. A method for identifying the gene product on which a compound which inhibits proliferation of an organism acts comprising: obtaining a culture comprising a plurality of strains wherein each strain underexpresses a different gene product which is essential for proliferation of said organism and wherein the nucleotide sequence of each of the underexpressed genes has been altered so as to include a nucleotide sequence which can be used to generate a unique product corresponding to each of the underexpressed genes, wherein said culture comprises a strain in which a gene product comprises a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is underexpressed; contacting said culture with a sufficient concentration of said compound to inhibit the proliferation of strains of said organism which underexpress said gene product on which said compound acts, such that strains which underexpress said gene product on which said compound acts proliferate more slowly than strains which do not underexpress the gene product on which said compound acts; and identifying the gene product which is underexpressed in a strain which proliferated more rapidly in said culture by detecting the unique product corresponding to said gene.

478. The method of Paragraph 472,473,474,475,476 or 477, wherein the nucleotide sequence of each of the genes encoding an underexpressed gene product has been altered by replacing the native promoters of said genes with promoters which facilitate underexpression of said gene products.

479. The method of Paragraph 472,473,474,475,476 or 477, wherein the nucleotide sequence of each of the genes encoding an underexpressed gene product has been altered by inserting a regulatory element into the native promoters of said genes with a promoter which facilitates underexpression of said gene products.

480. The method of Paragraph 479, wherein said regulatory element is selected from the group consisting of a regulatable promoter, an operator which is recognized by a repressor, a nucleotide sequence which is recognized by a transcriptional activator, a transcriptional terminator, a nucleotide sequence which introduces a bend in the DNA and an upstream activating sequence.

481. The method of Paragraph 472,473,474,475,476 or 477, wherein the step of identifying the gene product which is underexpressed in a strain which proliferated more slowly in said culture by detecting the unique product corresponding to said gene comprises performing an amplification reaction and detecting a unique amplification product corresponding to said gene.

482. The method of Paragraph 478, wherein the native promoter of each of the genes encoding a gene product essential for proliferation is replaced with the same promoter.

483. The method of Paragraph 478, wherein the native promoters of the genes encoding gene products essential for proliferation are replaced with a plurality of promoters selected to give a desired expression level for each gene product.

484. The method of Paragraph 478, wherein said promoters which replaced the native promoters in each strain comprise regulatable promoters.

485. The method of Paragraph 478, wherein said promoters which replaced the native promoters in each strain each strain comprise constitutive promoters.

486. The method of Paragraph 472,473,474,475,476 or 477, wherein said organism is selected from the group consisting of bacteria, fungi, and protozoa.

487. The method of Paragraph 472,473,474,475,476 or 477, wherein said culture is a culture of an organism selected from the group consisting of Acinetobacter bamnanaii, Anaplasfna rnarginale, Aspergillus furnigatus, Bacillus anthracis, Bacteroides fiagilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faeciuyn, Esc7zerichia coli, Haemophilus influenzae, helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Morazella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneurnocystis carinii, Proteus mirabilis, Proteus vulgaris, pseudomonas aeruginosa, Pseudomonas putida, pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella soranei, Staplzylococcus aureus, Staphylococcus epidernzidis, Staphylococcus haemolyticus, Streptococcus pneuynoniae, Streptococcus mutans, Streptococcus pyogefzes, Trepon. efna pallidum, Ureaplas7na urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis and any species falling within the genera of any of the above species.

488. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or underexpressed; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction.

489. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 is overexpressed or underexpressed ; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction.

490. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is

overexpressed or underexpressed; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; and determining the lengths of the amplification products obtained in said amplification reaction.

491. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least
70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-
6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed or underexpressed ; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction.

492. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.:
6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction.

493. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism, wherein said culture

comprises a strain in which a gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version
3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed or underexpressed; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction.

494. The method of Paragraph 488,489,490,491,492 or 493, wherein one member of each primer pair for each of said genes is labeled with a detectable dye.

495. The method of Paragraph 488,489,490,491,492 or 493, wherein: said nucleic acid sample is divided into N aliquots; and said amplification reaction is performed on each aliquot using primer pairs complementary to nucleotide sequences within or adjacent to 1/N of the genes which encode said gene products, wherein one of the members of each primer pair in each aliquot is labeled with a dye and wherein the dyes on the primers in each aliquot are distinguishable from one another.

496. The method of Paragraph 494, further comprising pooling the amplification products from each of the aliquots prior to determining the lengths of the amplification products.

497. The method of Paragraph 488,489,490,491,492 or 493, wherein the native promoters of said genes which encode said gene products have been replaced with a regulatable promoter and one of the primers in said primer pairs is complementary to a nucleotide sequence within said regulatable promoter.

498. The method of Paragraph 496, wherein the native promoters for each of said genes were replaced with the same regulatable promoter.

499. The method of Paragraph 496, wherein more than one regulatable promoter was used to replace the promoters of said genes such that some of said genes are under the control of a different regulatable promoter.

500. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising : obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound; obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound; performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction; and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or underexpressed.

501. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising: obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound ; obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains. has not been contacted with said compound; performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction; and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected fiom the group consisting of SEQ
ID NOs.: 6214-42397 is overexpressed or underexpressed.

502. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising: obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound; obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound; performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction; and comparing the amount of each amplification product in said first amplificatiuon reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed.

* 503. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising : obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound; obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound; performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs

which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction; and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:
1-6213 is overexpressed or underexpressed.

504. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising: obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound; obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound; performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction; and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second cultures or collection of strains comprise a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397

under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed.

505. A method for identifying the target of a compound which inhibits the proliferation of an organism comprising: obtaining a first nucleic acid sample comprising nucleic acids from a first culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains wherein each strain overexpresses or underexpresses a different gene product which is required for proliferation of said organism and wherein said culture or collection of strains has been contacted with said compound; obtaining a second nucleic acid sample comprising nucleic acids from a second culture or collection of strains wherein said culture or collection of strains comprises the same strains as said first culture or collection of strains wherein said second culture or collection of strains has not been contacted with said compound; performing a first amplification reaction on said first nucleic acid sample using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; performing a second amplification reaction on said second nucleic acid sample using the same set of primer pairs used in said first amplification reaction; and comparing the amount of each amplification product in said first amplification reaction to the amount of that amplification product in said second amplification reaction, wherein an increased level of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products and a decreased level of of an amplification product in said first amplification reaction relative to said second amplification reaction indicates that the gene product corresponding to said amplification product is the target of said compound if said culture or strain overexpresses said gene products, wherein said first and second culture or collection of strains comprise a strain in which a gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938- 78581 is overexpressed or underexpressed.

506. The method of Paragraph 500,501,502,503,504 or 505, wherein one member of each primer pair for each of said genes is labeled with a detectable dye.

507. The method of Paragraph 500,501,502,503,504 or 505, wherein the native promoters of said genes which encode said gene products have been replaced with a regulatable promoter and one of the primers in said primer pairs is complementary to a nucleotide sequence within said regulatable promoter.

508. The method of Paragraph 500,501,502,503,504 or 505, wherein the native promoters for each of said genes were replaced with the same regulatable promoter.

509. The method of Paragraph 500,501,502,503,504 or 505, wherein more than one regulatable promoter was used to replace the promoters of said genes such that some of said genes are under the control of a different regulatable promoter.

510. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or underexpressed.

511. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 6214-42397 is overexpressed or underexpressed.

512. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed.

513. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs:
1-6213 is overexpressed or underexpressed.

514. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which

transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS. : 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed.

515. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which transcribe an antisense nucleic acid complementary to a different gene product which is required for proliferation of said organism; performing an amplification reaction using a set of primer pairs which are complementary to nucleotide sequences within or adjacent to the nucleic acids which encode said antisense nucleic acids, wherein the members of said set of primer pairs are designed such that each primer pair would yield an amplification product having a length distinguishable from the lengths of the amplification products from the other primer pairs if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; and determining the lengths of the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938 78581 is overexpressed or underexpressed.

516. The method of Paragraph 510,511,512,513,514 or 515, wherein one member of each primer pair for each of said genes is labeled with a detectable dye.

517. The method of Paragraph 510,511,512,513,514 or 515, wherein: said nucleic acid sample is divided into N aliquots; and said amplification reaction is performed on each aliquot using primer pairs complementary to nucleotide sequences within or adjacent to 1/N of the genes which encode said gene products, wherein one of the members of each primer pair in each aliquot is labeled with a dye and wherein the dyes on the primers in each aliquot are distinguishable from one another.

518. The method of Paragraph 517, further comprising pooling the amplification products from each of the aliquots prior to determining the lengths of the amplification products.

519. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism; performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 is overexpressed or underexpressed.

520. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism; performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.:
6214-42397 is overexpressed or underexpressed.

521. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism; performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; and identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed.

522. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism; performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; and identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 1-
6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs : 1-6213 is overexpressed or underexpressed.

523. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising: obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism; performing an amplification reaction using primer

pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains ; and identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of
SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed.

524. A method for determining the extent to which each of a plurality of strains are present in a culture or collection of strains comprising : obtaining a nucleic acid sample comprising nucleic acids from a culture or collection of strains wherein said culture or collection of strains comprises a plurality of strains which overexpress or underexpress a different gene product which is required for proliferation of said organism; performing an amplification reaction using primer pairs which are complementary to nucleotide sequences within or adjacent to the genes which encode said gene products, wherein said primer pairs are designed such that each primer pair would yield an amplification product which is distinguishable from the amplification products produced by the other primer pairs on the a basis selected from the group consisting of length, detectable label and both length and detectable label if a strain comprising the nucleotide sequences complementary to said primer pair is present in said culture or collection of strains; and identifying the amplification products obtained in said amplification reaction, wherein said culture comprises a strain in which a gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938 78581 is overexpressed or underexpressed.

525. The method of Paragraph 519,520,521,522,523 or 524, wherein said primer pairs are divided into at least two sets, each primer pair comprises a primer which is labeled with a distinguishable dye, and the distinguishable dye used to label each set of primer pairs is distinguishable from the dye used to label the other sets of primer pairs.

526. The method of Paragraph 519,520,521,522,523 or 524, wherein: said nucleic acid sample is divided into N aliquots; and said amplification reaction is performed on each aliquot using primer pairs complementary to nucleotide sequences within or adjacent to 1/N of the genes which encode said gene products, wherein one of the members of each primer pair in each aliquot is labeled with a dye and wherein the dyes on the primers in each aliquot are distinguishable from one another.

527. The method of Paragraph 526, further comprising pooling the amplification products from each of the aliquots prior to determining the lengths of the amplification products.

528. The method of Paragraph 519,520,521,522,523 or 524, wherein the native promoters of said genes which encode said gene products have been replaced with a regulatable promoter and one of the primers in said primer pairs is complementary to a nucleotide sequence within said regulatable promoter.

529. The method of Paragraph 528, wherein the native promoters for each of said genes were replaced with the same regulatable promoter.

530. The method of Paragraph 528, wherein more than one regulatable promoter was used to replace the promoters of said genes such that some of said genes are under the control of a different regulatable promoter.

Definitions

By"biological pathway"is meant any discrete cell function or process that is carried out by a gene product or a subset of gene products. Biological pathways include anabolic, catabolic, enzymatic, biochemical and metabolic pathways as well as pathways involved in the production of cellular structures such as cell walls. Biological pathways that are usually required for proliferation of cells or microorganisms include, but are not limited to, cell division, DNA synthesis and replication, RNA synthesis (transcription), protein synthesis (translation), protein processing, protein transport, fatty acid biosynthesis, electron transport chains, cell wall synthesis, cell membrane production, synthesis and maintenance, and the like.

By"inhibit activity of a gene or gene product"is meant having the ability to interfere with the function of a gene or gene product in such a way as to decrease expression of the gene, in such a way as to reduce the level or activity of a product of the gene or in such a way as to inhibit the interaction of the gene or gene product with other biological molecules required for its activity.

Agents which inhibit the activity of a gene include agents that inhibit transcription of the gene, agents that inhibit processing of the transcript of the gene, agents that reduce the stability of the transcript of the gene, and agents that inhibit translation of the mRNA transcribed from the gene.

In microorganisms, agents which inhibit the activity of a gene can act to decrease expression of the operon in which the gene resides or alter the folding or processing of operon RNA so as to reduce the level or activity of the gene product. The gene product can be a non-translated RNA such as ribosomal RNA, a translated RNA (mRNA) or the protein product resulting from translation of the gene mRNA. Of particular utility to the present invention are antisense RNAs that have activities against the operons or genes to which they specifically hybridze.

By"activity against a gene product"is meant having the ability to inhibit the function or to reduce the level or activity of the gene product in a cell. This includes, but is not limited to, inhibiting the enzymatic activity of the gene product or the ability of the gene product to interact with other biological molecules required for its activity, including inhibiting the gene product's assembly into a multimeric structure.

By"activity against a protein"is meant having the ability to inhibit the function or to reduce the level or activity of the protein in a cell. This includes, but is not limited to, inhibiting the enzymatic activity of the protein or the ability of the protein to interact with other biological molecules required for its activity, including inhibiting the protein's assembly into a multimeric structure.

By"activity against a nucleic acid"is meant having the ability to inhibit the function or to reduce the level or activity of the nucleic acid in a cell. This includes, but is not limited to, inhibiting the ability of the nucleic acid interact with other biological molecules required for its activity, including inhibiting the nucleic acid's assembly into a multimeric structure.

By"activity against a gene"is meant having the ability to inhibit the function or expression of the gene in a cell. This includes, but is not limited to, inhibiting the ability of the gene to interact with other biological molecules required for its activity.

By"activity against an operon"is meant having the ability to inhibit the function or reduce the level of one or more products of the operon in a cell. This includes, but is not limited to, inhibiting the enzymatic activity of one or more products of the operon or the ability of one or more products of the operon to interact with other biological molecules required for its activity.

By"antibiotic"is meant an agent which inhibits the proliferation of a cell or microorganism.

By"E. coli or Eschericlaia coli"is meant Esc7z. erichia coli or any organism previously categorized as a species of Shigella including Shigella boydii, Shigella f7exiaeri, Shigella dysenteriae, Slzigella sonnei, Shigella 2A.

By"homologous coding nucleic acid"is meant a nucleic acid homologous to a nucleic acid encoding a gene product whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 or a portion thereof. In some embodiments, the homologous coding nucleic acid may have at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from

the group consisting of SEQ ID NOS.: 6214-42,397 and fragments comprising at least 10,15,20,25, 30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides thereof. In other embodiments the homologous coding nucleic acids may have at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of the nucleotide sequences complementary to one of SEQ ID NOs.: 1-6213 and fragments comprising at least 10,15,20,25,30,35,40,50,75,100,150, 200,300,400, or 500 consecutive nucleotides thereof. Identity may be measured using BLASTN version 2.0 with the default parameters or tBLASTX with the default parameters. (Altschul, S. F. et al. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs,
Nucleic Acid Res. 25: 3389-3402 (1997). Alternatively a"homologuous coding nucleic acid" could be identified by membership of the gene of interest to a functional orthologue cluster. All other members of that orthologue cluster would be considered homologues. Such a library of functional orthologue clusters can be found at http://www. ncbi. nlm. nih. gov/COG. A gene can be classified into a cluster of orthologous groups or COG by using the COGNITOR program available at the above web site, or by direct BLASTP comparison of the gene of interest to the members of the COGs and analysis of these results as described by Tatusov, R. L., Galperin, M. Y., Natale, D. A. and Koonin, E. V. (2000) The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Research v. 28 n. 1, pp33-36.

Homologous coding nucleic acids and the homologous polypeptides which they encode may also be identified using a"reciprocal"best-hit analysis. To facilitate the identification of homologous coding nucleic acids and homologous polypeptides, paralogous genes within each of 51 organisms are identified and clustered prior to comparison to other organisms. Briefly, the polypeptide sequence of each polypeptide encoded by each open reading frame (ORF) in a given organism is compared to the polypeptide sequence encoded by every other ORF for that organism for each of the 51 pathogenic organisms (PathoSeq Sept 2001 release) using BLASTP 2.09 algorithm without filtering. Simultaneously, the polypeptide sequence encoded by each ORF of an organism is compared to the polypeptide sequences encoded by each of the ORFs in the remaining 51 organisms. Those polypeptides within a single organism that shared a higher degree of sequence identity to one another than to polypeptide sequences obtained from any other organisms are clustered as"paralog"sequences for"reciprocal"best-hit analysis.

For each reference organism, the 50 homologous coding nucleic acids (and the 50 homologous polypeptides which they encode) can be determined by identifying the ORFs in each of the 50 comparison organisms which encode a polypeptide sharing the highest degree of amino acid sequence identity to the polypeptide encoded by the ORF from the reference organism. The accuracy of the identification of the predicted homologous coding nucleic acids (and the homologous polypeptides which they encode) is confirmed by a"reciprocal"BLAST analysis in which the polypeptide sequence of the predicted homologous polypeptide is compared against the polypeptides encoded by each of the ORFS in the reference organism using BLASTP 2.09 algorithm without filtering. Only those polypeptides that share the highest degree of amino acid sequence identity in each portion of the two-way comparison are retained for further analysis.

The term"homologous coding nucleic acid"also includes nucleic acids comprising nucleotide sequences which encode polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% maino acid identity or similarity to a polypeptide comprising the amino acid sequence of one of SEQ ID NOs: 42,398-78,581 or to a polypeptpide whose expression is inhibited by a nucleic acid comprising a nucleotide sequence of one of SEQ ID NOs: 1-6213 or fragments comprising at least 5,10,15,20, 25,30,35,40,50,75,100, or 150 consecutive amino acids thereof as determined using the FASTA version 3.0t78 algorithm with the default parameters. Alternatively, protein identity or similarity may be identified using BLASTP with the default parameters, BLASTX with the default parameters, TBLASTN with the default parameters, or tBLASTX with the default parameters.

(Altschul, S. F. et al. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database
Search Programs, Nucleic Acid Res. 25: 3389-3402 (1997).

Additionally, homologous coding nucleic acids and the homologous polypeptides which they encode may be identified using a"reciprocal"best-hit analysis. To facilitate the identification of homologous coding nucleic acids and homologous polypeptides, paralogous genes within each of 51 organisms are identified and clustered prior to comparison to other organisms. Briefly, the polypeptide sequence of each polypeptide encoded by each open reading frame

(ORF) in a given organism is compared to the polypeptide sequence encoded by every other ORF for that organism for each of the 51 pathogenic organisms (PathoSeq Sept 2001 release) using BLASTP 2.09 algorithm without filtering. Simultaneously, the polypeptide sequence encoded by each ORF of an organism is compared to the polypeptide sequences encoded by each of the ORFs in the remaining 51 organisms. Those polypeptides within a single organism that shared a higher degree of sequence identity to one another than to polypeptide sequences obtained from any other organisms are clustered as"paralog"sequences for"reciprocal"best-hit analysis.

For each reference organism, the 50 homologous coding nucleic acids (and the 50 homologous polypeptides which they encode) can be determined by identifying the ORFs in each of the 50 comparison organisms which encode a polypeptide sharing the highest degree of amino acid sequence identity to the polypeptide encoded by the ORF from the reference organism. The accuracy of the identification of the predicted homologous coding nucleic acids (and the homologous polypeptides which they encode) is confirmed by a"reciprocal"BLAST analysis in which the polypeptide sequence of the predicted homologous polypeptide is compared against the polypeptides encoded by each of the ORFS in the reference organism using BLASTP 2.09 algorithm without filtering. Only those polypeptides that share the highest degree of amino acid sequence identity in each portion of the two-way comparison are retained for further analysis.

The term"homologous coding nucleic acid"also includes coding nucleic acids which hybridize under stringent conditions to a nucleic acid selected from the group consisting of the nucleotide sequences complementary to one of SEQ ID NOS.: 6214-42,397 and coding nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of the sequences complementary to one of SEQ ID NOS.: 6214-42,397. As used herein,"stringent conditions"means hybridization to filter-bound nucleic acid in 6xSSC at about 45 C followed by one or more washes in 0. 1xSSC/0. 2% SDS at about 68 C. Other exemplary stringent conditions may refer, e. g., to washing in 6xSSC/0.05% sodium pyrophosphate at 37 C, 48 C, 55 C, and 60 C as appropriate for the particular probe being used.

The term"homologous coding nucleic acid"also includes coding nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleotide sequence selected from the group consisting of the sequences complementary to one of SEQ ID NOS.: 6214-42,397 and coding nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300, 400, or 500 consecutive nucleotides of the sequences complementary to one of SEQ ID NOS.: 6214-42,397. As used herein,"moderate conditions"means hybridization to filter-bound DNA in 6x sodium chloride/sodium citrate (SSC) at about 45 C followed by one or more washes in 0.2xSSC/0.1% SDS at about 42-65 C.

The tenn"homologous coding nucleic acids"also includes nucleic acids comprising nucleotide sequences which encode a gene product whose activity may be complemented by a gene encoding a gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213. In some embodiments, the homologous coding nucleic acids may encode a gene product whose activity is complemented by the gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42,397. In other embodiments, the homologous coding nucleic acids may comprise a nucleotide sequence encode a gene product whose activity is complemented by one of the polypeptides of SEQ ID NOs. 42,398-78,581.

The term"homologous antisense nucleic acid"includes nucleic acids comprising a nucleotide sequence having at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of one of the sequences of SEQ ID NOS. 1-6213 and fragments comprising at least 10,15,20,25, 30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides thereof. Homologous antisense nucleic acids may also comprising nucleotide sequences which have at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of the sequences complementary to one of sequences of SEQ ID NOS.: 6214-42,397 and fragments comprising at least 10,15,20,25,30,35, 40,50,75,100,150,200,300,400, or 500 consecutive nucleotides thereof. Nucleic acid identity may be determined as described above.

The term"homologous antisense nucleic acid"also includes antisense nucleic acids comprising nucleotide sequences which hybridize under stringent

conditions to a nucleotide sequence complementary to one of SEQ ID NOs.: 1-6213 and antisense nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of the sequence complementary to one of SEQ ID NOs. 1-6213. Homologous antisense nucleic acids also include antisense nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a nucleotide sequence selected fiom the group consisting of SEQ ID NOS.: 621442,397 and antisense nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10, 15,20,25,30,35,40,50,75,100,150,200, 300,400, or 500 consecutive nucleotides of one of SEQ ID NOS. : 6214-42, 397.

The term"homologous antisense nucleic acid"also includes antisense nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleotide sequence complementary to one of SEQ ID NOs.: 1-6213 and antisense nucleic acids comprising nucleotide seuqences which hybridize under moderate conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of the sequence complementary to one of SEQ ID NOs. 1-6213. Homologous antisense nucleic acids also include antisense nucleic acids comprising nucleotide seuqences which hybridize under moderate conditions to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 621442,397 and antisense nucleic acids which comprising nucleotide sequences hybridize under moderate conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150, 200,300,400, or 500 consecutive nucleotides of one of SEQ ID NOS.: 6214-42,397.

By"homologous polypeptide"is meant a polypeptide homologous to a polypeptide whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 or by a homologous antisense nucleic acid. The term "homologous polypeptide"includes polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a polypeptide whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs: 1-6213 or by a homologous antisense nucleic acid, or polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a polypeptide to a fragment comprising at least 5,10,15,20,25,30,35,40,50,75,100, or 150 consecutive amino acids of a polypeptide whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 or by a homologous antisense nucleic acid. Identity or similarity may be determined using the FASTA version 3.0t78 algorithm with the default parameters. Alternatively, protein identity or similarity may be identified using BLASTP with the default parameters, BLASTX with the default parameters, or TBLASTN with the default parameters. (Altschul, S. F. et al. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs, Nucleic Acid Res. 25: 3389-3402 (1997). Additionally, homologous coding nucleic acids and the homologous polypeptides which they encode may be identified using a "reciprocal"best-hit analysis. To facilitate the identification of homologous coding nucleic acids and homologous polypeptides, paralogous genes within each of 51 organisms are identified and clustered prior to comparison to other organisms. Briefly, the polypeptide sequence of each polypeptide encoded by each open reading frame (ORF) in a given organism is compared to the polypeptide sequence encoded by every other ORF for that organism for each of the 51 pathogenic organisms (PathoSeq Sept 2001 release) using BLASTP 2.09 algorithm without filtering.

Simultaneously, the polypeptide sequence encoded by each ORF of an organism is compared to the polypeptide sequences encoded by each of the ORFs in the remaining 51 organisms. Those polypeptides within a single organism that shared a higher degree of sequence identity to one another than to polypeptide sequences obtained from any other organisms are clustered as"paralog" sequences for"reciprocal"best-hit analysis.

For each reference organism, the 50 homologous coding nucleic acids (and the 50 homologous polypeptides which they encode) can be determined by identifying the ORFs in each of the 50 comparison organisms which encode a polypeptide sharing the highest degree of amino acid sequence identity to the polypeptide encoded by the ORF from the reference organism. The accuracy of the identification of the predicted homologous coding nucleic acids (and the homologous polypeptides which they encode) is confirmed by a"reciprocal"BLAST analysis in which the polypeptide sequence of the predicted homologous polypeptide is compared against the polypeptides encoded by each of the ORFS in the reference organism using BLASTP 2.09 algorithm without filtering. Only those polypeptides that share the highest degree of amino acid sequence identity in each portion of the two-way comparison are retained for further analysis.

The term homologous polypeptide also includes polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a polypeptide selected from the group consisting of SEQ ID NOs: 42,398-78,581 and polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a fragment comprising at least 5,10,15,20,25,30,35,40,50,75,100, or 150 consecutive amino acids of a polypeptide selected from the group consisting of SEQ ID NOs: 42,398-78,581.

The invention also includes polynucleotides, preferably DNA molecules, that hybridize to one of the nucleic acids of SEQ ID NOs.: 1-6213, SEQ ID NOs.: 6214-42,397 or the complements of any of the preceding nucleic acids. Such hybridization may be under stringent or moderate conditions as defined above or under other conditions which permit specific hybridization. The nucleic acid molecules of the invention that hybridize to these DNA sequences include oligodeoxynucleotides ("oligos") which hybridize to the target gene under highly stringent or stringent conditions. In general, for oligos between 14 and 70 nucleotides in length the melting temperature (Tm) is calculated using the formula:
Tm (C) = 81. 5 + 16.6 (log [monovalent cations (molar)] + 0.41 (% G+C)- (500/N) where N is the length of the probe. If the hybridization is carried out in a solution containing formamide, the melting temperature may be calculated using the equation:
Tm (C) = 81.5 + 16.6 (log [monovalent cations (molar)] + 0.41 (% G+C)- (0.61) (% formamide)- (500/N) where N is the length of the probe. In general, hybridization is carried out at about 20-25 degrees below Tm (for DNA-DNA hybrids) or about 10-15 degrees below Tm (for RNA-DNA hybrids).

Other hybridization conditions are apparent to those of slcill in the art (see, for example,
Ausubel, F. M. et al., eds., 1989, Current Protocols in Molecular Biology, Vol. I, Green Publishing
Associates, Inc. and John Wiley & Sons, Inc., New York, at pp. 6. 3.1-6.3.6 and 2.10.3.

The term, Salinoizella, is the generic name for a large group of gram negative enteric bacteria that are closely related to Escherichia coli. The diseases caused by Salmonella are often due to contamination of foodstuffs or the water supply and affect millions of people each year.

Traditional methods of Salmonella taxonomy were based on assigning a separate species name to each serologically distinguishable strain (Kauffmann, F 1966 The bacteriology of the
Enterobacteriaceae. Munlcsgaard, Copenhagen). Serology of Salo7ozella is based on surface antigens (O [somatic] and H [flagellar]). Over 2,400 serotypes or serovars of Sal7no71ella are known (Popoff, et al. 2000 Res. Microbiol. 151: 63-65). Therefore, each serotype was considered to be a separate species and often given names, accordingly (e. g. S. paratyphi, S. typhimurium, S. typhi, S. enteriditis, etc.).

However, by the 1970s and 1980s it was recognized that this system was not only cumbersome, but also inaccurate. Then, many Salmonella species were lumped into a single species (all serotypes and subgenera I, II, and IV and all serotypes of Arizona) with a second subspecies, S. oMgorn also recognized (Crosa, et al., 1973, J. Bacteriol. 115: 307-315). Though species designations are based on the highly variable surface antigens, the Salmonella are very similar otherwise with a major exception being pathogenicity determinants.

There has been some debate on the correct name for the Salmonella species. Currently (Brenner, et al. 2000 J. Clin. Microbiol. 38: 2465-2467), the accepted name is Salfnonella enterica.

S. enterica is divided into six subspecies (I, S. enterica subsp. enterica ; II, S. enterica, subsp. sala7nae ; IIIa, S. enterica subsp. arizona ; IIIb, S. enterica subsp. diarizonae ; IV, S. eiiterica subsp. houtenae ; and VI, S. enterica subsp. indica). Within subspecies I, serotypes are used to distinguish each of the serotypes or serovars (e. g. S. enterica serotype Enteriditis, S. enterica serotype
Typhimurium, S. enterica serotype Typhi, and S. enterica serotype Choleraesuis, etc.). Current convention is to spell this out on first usage (Salmonella enterica ser. Typhimurium) and then use an abbreviated form (Salnioraella Typhimurium or S. Typhimurium). Note, the genus and species names (Salmonella enterica) are italicized but not the serotype/serovar name (Typhimurium).

Because the taxonomic committees have yet to officially approve of the actual species name, this latter system is what is employed by the CDC (Brenner, et al. 2000 J. Clin. Microbiol. 38 : 24652467). Due to the concerns of both taxonomic priority and medical importance, some of these serotypes might ultimately receive full species designations (S. typhi would be the most notable).

Therefore, as used herein"Salmonella enterica or S. enterica"includes serovars Typhi,
Typhimurium, Paratyphi, Choleraesuis, etc."However, appeals of the"official"name are in process and the taxonomic designations may change (S. choleraesuis is the species name that could replace S. enterica based solely on priority).

By"identifying a compound"is meant to screen one or more compounds in a collection of compounds such as a combinatorial chemical library or other library of chemical compounds or to characterize a single compound by testing the compound in a given assay and determining whether it exhibits the desired activity.

By"inducer"is meant an agent or solution which, when placed in contact with a cell or microorganism, increases transcription, or inhibitor and/or promoter clearance/fidelity, from a desired promoter.

As used herein,"nucleic acid"means DNA, RNA, or modified nucleic acids. Thus, the terminology"the nucleic acid of SEQ ID NO: X"or"the nucleic acid comprising the nucleotide sequence"includes both the DNA sequence of SEQ ID NO: X and an RNA sequence in which the thymidines in the DNA sequence have been substituted with uridines in the RNA sequence and in which the deoxyribose backbone of the DNA sequence has been substituted with a ribose backbone in the RNA sequence. Modified nucleic acids are nucleic acids having nucleotides or structures which do not occur in nature, such as nucleic acids in which the internucleotide phosphate residues with methylphosphonates, phosphorothioates, phosphoramidates, and phosphate esters.

Nonphosphate internucleotide analogs such as siloxane bridges, carbonate brides, thioester bridges, as well as many others known in the art may also be used in modified nucleic acids. Modified nucleic acids may also comprise, a-anomeric nucleotide units and modified nucleotides such as 1,2dideoxy-d-ribofuranose, 1,2-dideoxy-1-phenylribofuranose, and A, N4-ethano-5-methyl-cytosine are contemplated for use in the present invention. Modified nucleic acids may also be peptide nucleic acids in which the entire deoxyribose-phosphate baclcbone has been exchanged with a chemically completely different, but structurally homologous, polyamide (peptide) baclcbone containing 2-aminoethyl glycine units.

As used herein,"sub-lethal"means a concentration of an agent below the concentration required to inhibit all cell growth.

Brief Description of the Drawings

Figure 1A illustrates a method for replacing a promoter using a promoter replacement cassette comprising a 5'region homologous to the sequence which is 5'of the natural promoter in the chromosome, the promoter which is to replace the chromosomal promoter and a 3'region which is homologous to sequences 3'of the natural promoter in the chromosome.

Figure 1B illustrates a method for replacing a promoter using a promoter replacement cassette comprising a nucleic acid encoding an identifiable or selectable marker disposed between the 5'region which is homologous to the sequence 5'of the natural promoter and the promoter which is to replace the chromosomal promoter and a transcriptional terminator 3'of the gene encoding an identifiable or selectable marlcer.

Figures 2A and 2B illustrate one method for identifying amplification products which are underrepresented or overrepresented in a culture.

Figures 3A and 3B illustrate another method for identifying amplification products which are underrepresented or overrepresented in a culture.

Figure 4 illustrates the results of a hybridization analysis where the antisense nucleic acid expressed by a strain in the culture is not complementary to all or a portion of the gene encoding the target of the compound (i. e. a nonspecific strain).

Figure 5 illustrates the results of a hybridization analysis where the antisense nucleic acid expressed by a strain in the culture is complementary to all or a portion of the gene encoding the target of the compound, the hybridization intensity for that strain will be intimately correlated with the concentration of the compound (i. e. a specific strain).

Figure 6 illustrates an oligonucleotide comprising a lac operator flanlced on each side by 40 nucleotides homologous to the promoter is the promoter which drives expression of the yabB yabC ftsL ftsl nzm E genes in an operon for use in inserting the lac operator into the promoter.

Figure 7 is an IPTG dose response curve in E. coli transformed with an IPTG-inducible plasmid containing either an antisense clone to the E. coli ribosomal protein rplW (AS-rplY) which is required for protein synthesis and essential for cell proliferation, or an antisense clone to the elaD (AS-elaD) gene which is not known to be involved in protein synthesis and which is also essential for proliferation.

Figure 8A is a tetracycline dose response curve in E. coli transformed with an IPTGinducible plasmid containing antisense to rplW (AS-rpl T) in the absence (0) or presence of IPTG at concentrations that result in 20% and 50% growth inhibition.

Figure 8B is a tetracycline dose response curve in E. coli transformed with an IPTGinducible plasmid containing antisense to elaD (AS-elaD) in the absence (0) or presence of IPTG at concentrations that result in 20% and 50% growth inhibition.

Figure 9 is a graph showing the fold increase in tetracycline sensitivity of E. coli transfected with antisense clones to essential ribosomal proteins L23 (AS-rplW) and L7/Ll2 and
L10 (AS-rplLrpl). Antisense clones to genes known to not be directly involved in protein synthesis, atpBlE (AS-atpBlE), visC (AS-visC), elaD (AS-elaD), yohH (AS-yohZ), are much less sensitive to tetracycline.

Figure 10 illustrates the results of an assay in which Staphylococcus aureus cells transcribing an antisense nucleic acid complementary to the gyrB gene encoding the P subunit of gyrase were contacted with several antibiotics whose targets were known.

Figure 11 illustrates a microtitration plate which contains antibiotic and inducer at gradient concentrations in a matrix format in 10 times excess quantity.

Figure 12 illustrates the results of an experiment demonstrating that at appropriate concentrations of inducer, cells which overexpress the defB gene product were able to grow at elevated concentrations of the antibiotic actinonin
Figure 13 illustrates the results of an experiment demonstrating that at appropriate concentrations of inducer cells which overexpress the folA gene product were able to grow at elevated concentrations of the antibiotic trimethoprim.

Figure 14 illustrates the results of an experiment demonstrating that overexpression of the fabI gene confers resistance to triclosan, which acts on the gene product of thefabl gene, but does not confer resistance to cerulenin, trimethoprim, or actinonin, each of which act on other gene products.

Figure 15 illustrates the results of an experiment demonstrating that overexpression of the folA gene confers resistance to trimethoprim, which acts on the gene product of the folA gene but does not confer resistance to triclosan, cerulenin, or actinonin, each of which act on other gene products.

Figure 16 illustrates the results of an experiment demonstrating that overexpression of the defB gene conferred resistance to actinonin, which acts on the gene product of the defB gene but does not confer resistance to cerulenin, trimethoprim, or triclosan, each of which act on other gene products.

Figure 17 illustrates the results of an experiment demonstrating that overexpression of the fail gene conferred resistance to cerulenin, which acts on the gene product of the & F gene, keto-acyl carrier protein synthase but does not confer resistance to triclosan, trimethoprim, or actinonin, each of which act on other gene products.

Figure 18 illustrates the results of experiments in which a mixture of nine strains was grown wells in a 96 well plate in medium containing various concentrations of inducer and a sufficient concentration of actinonin, cerulenin, triclosan or trimethoprim to inhibit the growth of strains which do not overexpress the targets of these antibiotics.

Detailed Description of Embodiments of the Invention

The present invention describes a group of prolcaryotic genes and gene families required for cellular proliferation. Exemplary genes and gene families from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkolderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacte cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticun are provided. A proliferation-required gene or gene family is one where, in the absence or substantial reduction of a gene transcript and/or gene product, growth or viability of the cell or microorganism is reduced or eliminated. Thus, as used herein, the terminology"proliferation-required"or"required for proliferation"encompasses instances where the absence or substantial reduction of a gene transcript and/or gene product completely eliminates cell growth as well as instances where the absence of a gene transcript and/or gene product merely reduces cell growth. These proliferation-required genes can be used as potential targets for the generation of new antimicrobial agents. To achieve that goal, the present invention also encompasses assays for analyzing proliferation-required genes and for identifying compounds which interact with the gene and/or gene products of the proliferation-required genes. In addition, the present invention contemplates the expression of genes and the purification of the proteins encoded by the nucleic acid sequences identified as required proliferation genes and reported herein. The purified proteins can be used to generate reagents and screen small molecule libraries or other candidate compound libraries for compounds that can be further developed to yield novel antimicrobial compounds.

The present invention also describes methods for identification of nucleotide sequences homologous to these genes and polypeptides described herein, including nucleic acids comprising nucleotide sequences homologous to the nucleic acids of SEQ ID NOS.: 6214-42397 and polypeptides homologous to the polypeptides of SEQ ID NOs.: 42398-78581. For example, these sequences may be used to identify homologous coding nucleic acids, homologous antisense nucleic acids, or homologous polypeptides in microorganisms such as Acinetobacter baumannii, Anaplasma marginale, Aspergillus furnigatus, Bacillus antlmacis, Bacteroides fi agilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida Irusei, Candida Itefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium Botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Eschenichia coli, Haernophilus influenze, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongo i, Salmoraella cholerasztis, Salmonella enterica, Salmonella paratyphi, Salmonella yplai, Salmonella typhirnurium, Slaigella boydii, S'laigella dysenteriae, Shigellaflexneri,

Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, . Ureaplasma urealyticunz, Vibrio cholerae, Vibrio parahaenaolyticus, Vibrio vulnificans, Yessinia enterocolitica, Yersifzia pestis or any species falling within the genera of any of the above species.

In some embodiments, the homologous coding nucleic acids, homologus antisense nucleic acids, or homologous polypeptides are identified in an organism other than E. coli.

The homologous coding nucleic acids, homologous antisense nucleic acids, or homologous polypeptides, may then be used in each of the methods described herein, including methods of identifying compounds which inhibit the proliferation of the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods of inhibiting the growth of the organism containing the homologous coding nucleic acid, homologus antisense nucleic acid or homologous polypeptide, methods of identifying compounds which influence the activity or level of a gene product required for proliferation of the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods for identifying compounds or nucleic acids having the ability to reduce the level or activity of a gene product required for proliferation of the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods of inhibiting the activity or expression of a gene in an operon required for proliferation of the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods for identifying a gene required for proliferation of the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods for identifying the biological pathway in which a gene or gene product required for proliferation of the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide lies, methods for identifying compounds having activity against biological pathway required for proliferation of the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods for determining the biological pathway on which a test compound acts in the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods of replacing an endogenous promoter with a regulatable promoter which controls the expression of the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods of inserting an operator within or near an endogenous promoter to provide regulatable expression of the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, methods of identifying the target on which a compound acts in the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide, and methods of inhibiting the proliferation of the organism containing the homologous coding nucleic acid, homologous antisense nucleic acid or homologous polypeptide in a subject. In some embodiments of the present invention, the methods are performed using an organism, other than E. coli or a gene or gene product from an organism other than E. coli.

One embodiment of the present invention utilizes a novel method to identify proliferationrequired sequences. Generally, a library of nucleic acid sequences from a given source are subcloned or otherwise inserted immediately downstream of an inducible promoter on an appropriate vector, such as a Staphylococcus aureus. coli or Pseudomonas aeruginosa/E. coli shuttle vector, or a vector which will replicate in both Salmonella typhimurium and Klebsiella pneumoniae, or other vector or shuttle vector capable of functioning in the intended organism, thus forming an expression library. It is generally preferred that expression is directed by a regulatable promoter sequence such that expression level can be adjusted by addition of variable concentrations of an inducer molecule or of an inhibitor molecule to the medium. For example, a number of regulatable promoters useful for regulating the expression of nucleic acid sequences over a wide range of expression levels are described in U. S.

Patent Application Serial Number 10/032,393, filed December 21,2001. Temperature activated promoters, such as promoters regulated by temperature sensitive repressors, such as the lambda Cissy repressor, are also envisioned. Although the insert nucleic acids may be derived from the chromosome of the cell or microorganism into which the expression vector is to be introduced, because the insert is not in its natural chromosomal location, the insert nucleic acid is an exogenous nucleic acid for the purposes of the discussion herein. The term"expression"is defined as the production of a sense or antisense RNA molecule from a gene, gene fragment, genomic fragment, chromosome, operon or portion thereof. Expression can also be used to refer to the process of peptide or polypeptide synthesis.

An expression vector is defined as a vehicle by which a ribonucleic acid (RNA) sequence is transcribed from a nucleic acid sequence carried within the expression vehicle. The expression vector can also contain features that permit translation of a protein product from the transcribed RNA message expressed from the exogenous nucleic acid sequence carried by the expression vector. Accordingly, an expression vector can produce an RNA molecule as its sole product or the expression vector can produce a RNA molecule that is ultimately translated into a protein product.

Once generated, the expression library containing the exogenous nucleic acid sequences is introduced into a population of cells (such as the organism from which the exogenous nucleic acid sequences were obtained) to search for genes that are required for bacterial proliferation. Because the library molecules are foreign, in context, to the population of cells, the expression vectors and the nucleic acid segments contained therein are considered exogenous nucleic acid.

Expression of the exogenous nucleic acid fragments in the test population of cells containing the expression library is then activated. Activation of the expression vectors consists of subjecting the cells containing the vectors to conditions that result in the expression of the exogenous nucleic acid sequences carried by the expression library. The test population of cells is then assayed to determine the effect of expressing the exogenous nucleic acid fragments on the test population of cells. Those expression vectors that negatively impact the growth of the cells upon induction of expression of the random sequences contained therein are identified, isolated, and purified for further study.

In some embodiments, vectors which comprises a regulatable fusion promoter selected from a suite of fusion promoters, wherein the promoter suite is useful for modulating both the basal and maximal levels of transcription of a nucleic acid over a wide dynamic range thus allowing the desired level of production of a transcript, can be used to express exogenous nucleic acids, including the nucleic acids of the present invention. Such promoters are described in U. S. Patent Application Serial Number 10/032,393, filed December 21,2001, the disclosure of which is incorported herein by reference in its entirety.

In some other embodiments, vectors useful for the production of stabilized mRNA having an increased lifetime (including antisense RNA) in Gram negative organisms are described in U. S.

Provisional Patent Application Serial Number 60/343,512, filed December 21,2001. Briefly, the stabilized antisense RNA may comprise an antisense RNA which was identified as inhibiting proliferation as described above which has been engineered to contain at least one stem loop flanking each end of the antisense nucleic acid. In some embodiments, the at least one stem-loop structure formed at the 5'end of the stabilized antisense nucleic acid comprises a flush, double stranded 5'end. In some embodiments, one or more of the stem loops comprises a rho independent terminator. In additional embodiments, the stabilized antisense RNA lacks a ribosome binding site.

In further embodiments, the stabilized RNA lacks sites which are cleaved by one or more RNAses, such as RNAse E or RNAse III. In some embodiments, the stabilized antisense RNA may be transcribed in a cell which the activity of at least one enzyme involved in RNA degradation has been reduced. For example, the activity of an enzyme such as RNase E, RNase II, RNase III, polynucleotide phosphorylase, and poly (A) polymerase, RNA helicase, enolase or an enzyme having similar functions may be reduced in the cell.

Alternatively, genes required for proliferation may be identified by replacing the natural promoter for the proliferation required gene with a regulatable promoter as described above. The growth of such strains under conditions in which the promoter is active or non-repressed is compared to the growth under conditions in which the promoter is inactive or repressed. If the strains fail to grow or grow at a substantially reduced rate under conditions in which the promoter is inactive or repressed but grow normally under conditions in which the promoter is active or nonrepressed, then the gene which is operably linked to the regulatable promoter encodes a gene product required for proliferation. For example, proliferation-required genes and gene products identified using promoter replacement are described in U. S. Patent Application Serial Number 09/948,993.

For example, in some embodiments, the natural promoter may be replaced using techniques which employ homologous recombination to exchange a promoter present on the chromosome of the cell with the desired promoter. In such methodology, a nucleic acid comprising a promoter replacement cassette is introduced into the cell. As illustrated in Figure 1A, the promoter replacement cassette comprises a 5'region homologous to the sequence which is 5'of the

natural promoter in the chromosome, the promoter which is to replace the chromosomal promoter and a 3' region which is homologous to sequences 3'of the natural promoter in the chromosome. In some embodiments, the promoter replacement cassette may also include a nucleic acid encoding an identifiable or selectable marker disposed between the 5'region which is homologous to the sequence 5'of the natural promoter and the promoter which is to replace the chromosomal promoter. If desired, the promoter replacement cassette may also contain a transcriptional terminator 3'of the gene encoding an identifiable or selectable marker, as illustrated in Figure 1B.

As illustrated in Figure 1A and 1B, homologous recombination is allowed to occur between the chromosomal region containing the natural promoter and the promoter replacement cassette. Cells in which the promoter replacement cassette has integrated into the chromosome are identified or selected. To confirm that homologous recombination has occurred, the chromosomal structure of the cells may be verified by Southern analysis or PCR.

In some embodiments, the promoter replacement cassette may be introduced into the cell as a linear nucleic acid, such a PCR product or a restriction fragment. Alternatively, the promoter replacement may be introduced into the cell on a plasmid. Figures 1A and 1B illustrates the replacement of a chromosomal promoter with a desired promoter through homologous recombination.

In some embodiments, the cell into which the promoter replacement cassette is introduced may carry mutations which enhance its ability to be transformed with linear DNA or which enhance the frequency of homologous recombination. For example, if the cell is an Escherichia coli cell it may have a mutation in the gene encoding Exonuclease V of the RecBCD recombination complex.

If the cell is an Escherichia coli cell it may have a mutation that activates the RecET recombinase of the Rac prophage and/or a mutation that enhances recombination through the RecF pathway. For example, the Escherichia coli cells may be RecB or RecC mutants carrying an sbcA or sbcB mutation. Alternatively, the Escherichia coli cells may be recD mutants. In other embodiments the
Escherichia coli cells may express the X Red recombination genes. For example, Escherichia coli cells suitable for use in techniques employing homologous recombination have been described in Datsenko, K. A. and Wanner, B. L., PNAS 97: 6640-6645 (2000); Murphy, K. C., J. Bact 180: 20532071 (1998); Zhang, Y., et al., Nature Genetics 20: 123-128 (1998); and Muyrers, J. P. P. et al.,
Genes & Development 14: 1971-1982 (2000). It will be appreciated that cells carrying mutations in similar genes may be constructed in organisms other than Escherichia coli.

In some embodiments of the present invention, a regulatable fusion promoter selected from a suite of fusion promoters, wherein the promoter suite is useful. for modulating both the basal and maximal levels of transcription of a nucleic acid over a wide dynamic range thus allowing the desired level of production of a transcript, is with the promoter replacement methods described above. Such promoters are described in U. S. Patent Application Serial Number 10/032,393, filed
December 21,2001, the disclosure of which is incorported herein by reference in its entirety.

A variety of assays are contemplated to identify nucleic acid sequences that negatively impact growth upon expression. In one embodiment, growth in cultures expressing exogenous nucleic acid sequences and growth in cultures not expressing these sequences is compared. Growth measurements are assayed by examining the extent of growth by measuring optical densities. Alternatively, enzymatic assays can be used to measure bacterial growth rates to identify exogenous nucleic acid sequences of interest. Colony size, colony morphology, and cell morphology are additional factors used to evaluate growth of the host cells. Those cultures that fail to grow or grow at a reduced rate under expression conditions are identified as containing an expression vector encoding a nucleic acid fragment that negatively affects a proliferation-required gene.

Once exogenous nucleic acids of interest are identified, they are analyzed. The first step of the analysis is to acquire the nucleotide sequence of the nucleic acid fragment of interest. To achieve this end, the insert in those expression vectors identified as containing a nucleotide sequence of interest is sequenced, using standard techniques well known in the art. The next step of the process is to determine the source of the nucleotide sequence. As used herein"source"means the genomic region containing the cloned fragment.

Determination of the gene (s) corresponding to the nucleotide sequence is achieved by comparing the obtained sequence data with databases containing grown protein and nucleotide sequences from various microorganisms. Thus, initial gene identification is made on the basis of significant sequence similarity or identity to either characterized or predicted Escherichia coli,
Staphylococcus aureus, Enterococcus faecalis, klebsiella pneumoniae, Pseudomonas aeruginosa, and Salmonella typhimurium genes or their encoded proteins and/or homologues in other species.

The number of nucleotide and protein sequences available in database systems has been growing exponentially for years. For example, the complete nucleotide sequences of Caenorhabditis elegant and several bacterial genomes, including E. coli, Aeropyrum pernix, Aquffex aeolicus,
Archaeoglobus fulgidus, Bacillus subtilis, Borrelia burgdorferi, Chlamydia pneunloniae,
Chlamydia trachomatis, Clostridium tetani, Corynebacterium diptheria, Deinococcus radiodurans,
Haemophilus i71fluenzae, Helicobacter pylori 26695, Helicobacter pylori J99, Methanobacterium therfnoautotrophicurn, Methanococcus jannaschii,
Mycobacteriunz tuberculosis, Mycoplasma genitalium, Mycoplasyna pneumoniae, Pseudoriaonas aeruginosa, Pyrococcus abyssi, Pyrococcus horikoshii,
Riclcettsia prowazelcii, Synechocystis PCC6803, Tlaerrnotoga maritima, Treponema pallidum, Bordetella pertussis, campylobacter jejuni, Clostridium acetobutylicum, Mycobacterium tuberculosis CSU#93, Neisseria gononrhoeae, Neisseria rze7zingitidis, Pseudornooaas aeruginosa,
Pyrobaculum aerophilum, Pyrococcus furiosus, Rhodobacter capsulatus, Salmonella (yphinzurium, Streptococcus mutans, Streptococcus pyogenes,
Ureaplasma urealyticuna and Vibrio cholera are available. This nucleotide sequence information is stored in a number of databanks, such as GenBank, the National Center for Biotechnology Information (NCBI), the Genome Sequencing Center (http://genome. wustl. edu/gsc/salmonella. shtml), and the Sanger Centre (http://www. sanger. ac. ulc/projects/S-typhi) which are publicly available for searching. A variety of computer programs are available to assist in the analysis of the sequences stored within these databases. FASTA, (W. R. Pearson (1990)"Rapid and Sensitive Sequence Comparison with
FASTP and FASTA"Methods in Enzymology 183: 63- 98), Sequence Retrieval System (SRS), (Etzold & Argos, SRS an indexing and retrieval tool for flat file data libraries. Comput. Appl.

Biosci. 9: 49-57,1993) are two examples of computer programs that can be used to analyze sequences of interest. In one embodiment of the present invention, the BLAST family of computer programs, which includes BLASTN version 2.0 with the default parameters, or BLASTX version 2.0 with the default parameters, is used to analyze nucleotide sequences.

BLAST, an acronym for"Basic Local Alignment Search Tool,"is a family of programs for database similarity searching. The BLAST family of programs includes: BLASTN, a nucleotide sequence database searching program, BLASTX, a protein database searching program where the input is a nucleic acid sequence; and BLASTP, a protein database searching program. BLAST programs embody a fast algorithm for sequence matching, rigorous statistical methods for judging the significance of matches, and various options for tailoring the program for special situations. Assistance in using the program can be obtained by e-mail at blast@ncbi. nlm. nih. gov. tBLASTX can be used to translate a nucleotide sequence in all three potential reading frames into an amino acid sequence.

Bacterial genes are often transcribed in polycistronic groups. These groups comprise operons, which are a collection of genes and intergenic sequences under common regulation. The genes of an operon are transcribed on the same mRNA and are often related functionally. Given the nature of the screening protocol, it is possible that the identified exogenous nucleic acid corresponds to a gene or portion thereof with or without adjacent noncoding sequences, an intragenic sequence (i. e. a sequence within a gene), an intergenic sequence (i. e. a sequence between genes), a nucleotide sequence spanning at least a portion of two or more genes, a 5'noncoding region or a 3'noncoding region located upstream or downstream from the actual nucleotide sequence that is required for bacterial proliferation.

Accordingly, it is often desirable to determine which gene (s) that is encoded within the operon is individually required for proliferation.

In one embodiment of the present invention, an operon is identified and then dissected to determine which gene or genes are required for proliferation.

Operons can be identified by a variety of means known to those in the art. For example, the RegulonDB DataBase described by Huerta et al. (Nucl. Acids Res. 26: 55-59,1998), which may also be found on the website http://www. cifn. unam. mx/Computational~Biology/regulondb/, provides information about operons in Escherichia coli. The Subtilist database (http://bioweb. pasteur. fr/GenoList/SubtiList), (Moszer, I., Glaser, P. and Danchin, A. (1995) Microbiology 141: 261-268 and Moszer, I (1998) FEBS Letters 430: 28-36, may also be used to predict operons. This database lists genes from the fully sequenced, Gram positive bacteria, Bacillus subtilis, together with predicted promoters and terminator sites. This information can be used in conjunction with the Staphylococcus aureus genomic sequence data to predict operons and thus produce a list of the genes affected by the antisense nucleic acids of the present invention. The Pseudomonas aeruginosa web site (http :// www. pseudomonas. com) can be used to help predict operon organization in this bacterium.

The databases available from the Genome Sequencing Center (http ://genome. wustl. edu/gsc/salmonella. shtml), and the Sanger Centre (http ://www. sanger. ac. uldprojects/S-typhi) may be used to predict operons in Salmonella typhimurium. The TIGR microbial database has an incomplete version of the E. faecalis genome http://www. tigr. org/cgi-bin/BlastSearch/blast. cgi? organism--e-faecalis. One can take a nucleotide sequence and BLAST it for homologs.

A number of techniques that are well known in the art can be used to dissect the operon.

Analysis of RNA transcripts by Northern blot or primer extension techniques are commonly used to analyze operon transcripts. In one aspect of this embodiment, gene disruption by homologous recombination is used to individually inactivate the genes of an operon that is thought to contain a gene required for proliferation.

Several gene disruption techniques have been described for the replacement of a functional gene with a mutated, non-functional (null) allele. These techniques generally involve the use of homologous recombination. One technique using homologous recombination in stapll ocöccus aureus is described in Xia et a.. 1999, Plasmid 42: 144-149. This technique uses crossover PCR to create a null allele with an in-frame deletion of the coding region of a target gene. The null allele is constructed in such a way that nucleotide sequences adjacent to the wild type gene are retained.

These homologous sequences surrounding the deletion null allele provide targets for homologous recombination so that the wild type gene on the Staphylococcus aureus chromosome can be replaced by the constructed null allele. This method can be used with other bacteria as well, including Salmonella and Klebsiella species. Similar gene disruption methods that employ the counter selectable marker sacB (Schweizer, H. P., Klassen, T. and Hoang, T. (1996) Mol. Biol. of Pseudomonas. ASM press, 229-237, are available for Pseudornonas, Salnioraella and Klebsiella species. E. faecalis genes can be disrupted by recombining in a non-replicating plasmid that contains an internal fragment to that gene (Leboeuf, C., L. Leblanc, Y. Auffray and A. Hartke.

2000. J. Bacteriol. 182: 5799-5806.

The crossover PCR amplification product is subcloned into a suitable vector having a selectable marker, such as a drug resistance marker. In some embodiments the vector may have an origin of replication which is functional in E. coli or another organism distinct from the organism in which homologous recombination is to occur, allowing the plasmid to be grown in E. coli or the organism other than that in which homologous recombination is to occur, but may lack an origin of replication functional in Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdo7fe7i, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytoge7les, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida,

Proteus mirabilis, Pseudomonas gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis such that selection of the selectable marker requires integration of the vector into the homologous region of the Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baufnanraii, Bacillus anthracis, Bacteroides fi agilis, Bordetella pertussis, Borrelia burgdorferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faeciuni, Haeiizophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococus epidermidis, Staphylococus haemolyticus, Streptococcus mutans, Streptococcus pneumonaie, Streptococcus pyogenes, Treponema pallidium, Ureaplasma urealyticum, Vibrio integration Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis chromosome. Usually a single crossover event is responsible for this
integration event such that the Escherichia coli, Staplzylococcus aureus, Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus antlzracis, Bacteroides fiagilis, Bordetella pertussis, Borrelia burgdorferi,
Burlclzolderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacteriurn diptlaeriae, Enterobacter cloacae, Enterococcus faeciuni, Haemophilus irzfluenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus
mirabilis, Pseudomonas putida, Pseudononas syringae, Salnaonella paratyplai, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneunloniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholera or Yersinia pestis chromosome now contains a tandem duplication of the target gene consisting of one wild type allele and one deletion null allele separated by vector sequence.

Subsequent resolution of the duplication results in both removal of the vector sequence and either restoration of the wild type gene or replacement by the in-frame deletion. The latter outcome will not occur if the gene should prove essential. A more detailed description of this method is provided in Example 10 below. It will be appreciated that this method may be practiced with any of the nucleic acids or organisms described herein.

Recombinant DNA techniques can be used to express the entire coding sequences of the gene identified as required for proliferation, or portions thereof. The over-expressed proteins can be used as reagents for further study. The identified exogenous sequences are isolated, purified, and cloned into a suitable expression vector using methods well grown in the art. If desired, the nucleic acids can contain the nucleotide sequences encoding a signal peptide to facilitate secretion of the expressed protein.

Expression of fragments of the bacterial genes identified as required for proliferation is also contemplated by the present invention. The fragments of the identified genes can encode a polypeptide comprising at least 5, at least 10, at least 15, at least 20, at least 25, at least 30 ; at least 35, at least 40, at least 45, at least 50, at least 55, at least 60, at least 65, at least 75, or more than 75 consecutive amino acids of a gene complementary to one of the identified sequences of the present invention. The nucleic acids inserted into the expression vectors can also contain endogenous sequences upstream and downstream of the coding sequence.

When expressing the encoded protein of the identified nucleic acid required for bacterial proliferation or a fragment thereof, the nucleic acid to be expressed is operably linked to a promoter in an expression vector using conventional cloning technology. The expression vector can be any of the bacterial, insect, yeast, or mammalian expression systems known in the art. Commercially available vectors and expression systems are available from a variety of suppliers including Genetics Institute (Cambridge, MA), Stratagene (La Jolla, California), Promega (Madison, Wisconsin), and Invitrogen (San Diego, California). If desired, to enhance expression and facilitate proper protein folding, the codon usage and codon bias of the sequence can be optimized for the particular expression organism in which the expression vector is introduced, as explained by Hatfield, et al., U. S. Patent No. 5,082,767, incorporated herein by this reference. Fusion protein expression systems are also contemplated by the present invention.

Following expression of the protein encoded by the identified exogenous nucleic acid, the protein may be purified. Protein purification techniques are well known in the art. Proteins encoded and expressed from identified exogenous nucleic acids can be partially purified using precipitation techniques, such as precipitation with polyethylene glycol. Alternatively, epitope tagging of the protein can be used to allow simple one step purification of the protein. In addition, chromatographic methods such as ion-exchange chromatography, gel filtration, use of hydroxyapaptite columns, immobilized reactive dyes, chromatofocusing, and use of high-performance liquid chromatography, may also be used to purify the protein. Electrophoretic methods such as one-dimensional gel electrophoresis, highresolution two-dimensional polyacrylamide electrophoresis, isoelectric focusing, and others are contemplated as purification methods. Also, affinity chromatographic methods, comprising antibody columns, ligand presenting columns and other affinity chromatographic matrices are contemplated as purification methods in the present invention.

The purified proteins produced from the gene encoding sequences identified as required for proliferation can be used in a variety of protocols to generate useful antimicrobial reagents. In one embodiment of the present invention, antibodies are generated against the proteins expressed from the identified exogenous nucleic acids. Both monoclonal and polyclonal antibodies can be generated against the expressed proteins. Methods for generating monoclonal and polyclonal antibodies are well known in the art. Also, antibody fragment preparations prepared from the produced antibodies discussed above are contemplated.

In addition, the purified protein, fragments thereof, or derivatives thereof may be administered to an individual in a pharmaceutically acceptable carrier to induce an immune response against the protein. Preferably, the immune response is a protective immune response which protects the individual. Methods for determining appropriate dosages of the protein and pharmaceutically acceptable carriers may be determined empiracally and are familiar to those skilled in the art.

Another application for the purified proteins of the present invention is to screen small molecule libraries for candidate compounds active against the various target proteins of the present invention. Advances in the field of combinatorial chemistry provide methods, well known in the art, to produce large numbers of candidate compounds that can have a binding, or otherwise inhibitory effect on a target protein. Accordingly, the screening of small molecule libraries for compounds with binding affinity or inhibitory activity for a target protein produced from an identified gene is contemplated by the present invention.

In some embodiments of the present invention, a cell sensitized by expressing an an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, an antisense nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100,150,200, 300,400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a nucleic acid complementary to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100,150, 200,300,400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of
SEQ ID NOs.: 42398-78581, a nucleic acid complementary to a nucleic acid which encodes at least 5,10,15,20,25,30,35,40,50,75,100, or 150 consecutive amino acids of a polypeptide sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a homologous antisense nucleic acid, an antisense nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100,150,200, 300,400, or 500 consecutive nucleotides of a homologous nucleic acid, a nucleic acid complementary to a homologous coding nucleic acid, a nucleic acid complementary to at least 10,

15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of a homologous coding nucleic acid, a nucleic acid complementary to a nucleic acid which encodes a homologous polypeptide, or a nucleic acid complementary to a nucleic acid which encodes at least 5,10,15,20,25,30,35,40,50,75,100, or 150 consecutive amino acids of a homologous polypeptide, is contacted with one or more candidate compounds from a small molecule library.

Candidate compounds which further inhibit the proliferation of the sensitized cell may be identified as possessing inhibitory activity for a target protein or product produced by the gene to which the antisense sequence is complementary.

A number of vectors useful in the above methods are described in U. S. Patent Application Serial Number 10/032,393, filed December 21,2001.

In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U. S. Patent Application Serial Number 60/343,512, can be used for the production of a stabilized transcript, which corresponds to a nucleic acid described herein, having an increased lifetime in Gram-negative organisms. Briefly, the stabilized antisense RNA may comprise an antisense RNA which was identified as inhibiting proliferation as described above which has been engineered to contain at least one stem loop Nanking each end of the antisense nucleic acid. In some embodiments, the at least one stem-loop structure formed at the 5'end of the stabilized antisense nucleic acid comprises a flush, double stranded 5'end. In some embodiments, one or more of the stem loops comprises a rho independent terminator. In additional embodiments, the stabilized antisense RNA lacks a ribosome binding site. In further embodiments, the stabilized RNA lacks sites which are cleaved by one or more RNAses, such as RNAse E or RNAse III. In some embodiments, the stabilized antisense RNA may be transcribed in a cell which the activity of at least one enzyme involved in RNA degradation has been reduced. For example, the activity of an enzyme such as RNase E, RNase II, RNase III, polynucleotide phosphorylase, and poly (A) polymerase, RNA helicase, enolase or an enzyme having similar functions may be reduced in the cell.

The present invention further contemplates utility against a variety of other pathogenic microorganisms in addition to Esclaerichia coli, Staplaylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia penumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridum botu1inum, Clostridiu7n difficile, Corynebacte7 ium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haefaophilus influenzae, Helicobacter pylori, Legionella pneunaophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacteriunt avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphyloccocus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidu7n, Ureaplasma urealyticum, Vibrio cholerae and Yersinia pestis. For example, homologous coding nucleic acids, homologous antisense nucleic acids or homologous polypeptides from other pathogenic microorganisms (including nucleic acids homologous to the nucleic acids of SEQ ID NOs.: 6214-42397, nucleic acids homologous to the antisense nucleic acids of SEQ ID NOs.: 1-6213, and polypeptides homologous to the polypeptides of SEQ ID NOs.: 42398-78581) may be identified using methods such as those described herein. The homologous coding nucleic acids, homologous antisense nucleic acids or homologous polypeptides may be used to identify compounds which inhibit the proliferation of these other pathogenic microorganisms using methods such as those described herein.

For example, the proliferation-required nucleic acids, antisense nucleic acids, and polypeptides from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salnzonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei,

Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacteriurn bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptoccus mutans, Streptoccus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis described herein (including the nucleic acids of SEQ ID NOs.: 6214-42397, the antisense nucleic acids of SEQ ID NOs: 1-6213, and the polypeptides of SEQ ID NOs.: 42398 78581) may be used to identify homologous coding nucleic acids, homologous antisense nucleic acids or homologous polypeptides required for proliferation in prokaryotes and eukalyotes. For example, nucleic acids or polypeptides required for the proliferation of protists, such as Plasmodium spp.; plants; animals, such as Eiztainoeba spp. and Contracaecum spp ; and fungi including Candida spp., (e. g., Candida albicans), Cryptococcus neoformans, and Aspergillus fumigatus may be identified. In one embodiment of the present invention, monera, specifically bacteria, including both Gram positive and Gram negative bacteria, are probed in search of novel gene sequences required for proliferation.

Likewise, homologous antisense nucleic acids which may be used to inhibit growth of these organisms or to identify antibiotics may also be identified. These embodiments are particularly important given the rise of drug resistant bacteria.

The number of bacterial species that are becoming resistant to existing antibiotics is growing.

A partial list of these microorganisms includes: Escherichia spp., such as E. coli, Enterococcus spp, such as E. faecalis ; Pseudomonas spp., such as P. aeruginosa, Clostridium spp., such as C. botulinum, Haemophilus spp., such as H. influenzae, Enterobacter spp., such as E. cloacae, Vibrio spp., such as V. cholera ; Moraxala spp., such as M. catarrhalis ; Streptococcus spp., such as S. pneumoniae, Neisseria spp., such as N. gonorrhoeae ; Mycoplasma spp., such as Mycoplasma pneumoniae ; Salmonella typhimuriu7n ; Helicobacterp) loi-i ; Escherichia coli ; and Mycobacterium tuberculosis. The genes and polypeptides identified as required for the proliferation of Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aerugi7aosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, CLostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumo7liae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the nucleic acids of SEQ ID NOs.: 621442397, the sequences complementary to the nucleic acids of SEQ ID NOs.: 6214-42397, and the polypeptides of SEQ ID NOs.: 42398-78581) can be used to identify homologous coding nucleic acids or homologous polypeptides required for proliferation from these and other organisms using methods such as nucleic acid hybridization and computer database analysis. Lilcewise, the antisense nucleic acids which inhibit proliferation of Esclzerichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacte7ium bovis,

Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptoccus mutans, Streptoccus pneumoniae, Streptoccus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the antisense nucleic acids of SEQ ID NOs.: 1-6213 or the sequences complementary thereto) may also be used to identify antisense nucleic acids which inhibit proliferation of these and other microorganisms or cells using nucleic acid hybridization or computer database analysis.

In one embodiment of the present invention, the nucleic acid sequences from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoraiae, Pseudornonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burlcholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haerraophilus iizfluenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidium, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the nucleic acids of SEQ ID NOs.: 621442397 and the antisense nucleic acids of SEQ ID NOs. 1-6213) are used to screen genomic libraries generated from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridiurn difficile, Cofynebacteriunz diptheriae, Enterobacter cloacae, Enterococcus faeciurn, Haemophilus influenzae, Helicobacter pylori, legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphyloccus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidium, Ureaplasma urealyticum, Vibrio cholerae, Yersinia pestis and other bacterial species of interest. For example, the genomic library may be from Gram positive bacteria, Gram negative bacteria or other organisms including Acinetobacter baurnannii, Anaplasma marginale, Aspergillus funigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlcholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida Icefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidiodies immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherchia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis,

Salmonella enterica, Salmonella paratyphi,
Salmoiaella typhi, Salmonella typhinauriurn, Shigella boydii, Slaigella dysenteriae, Shigella flexneri,
Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificalls, Yersinia enterocolitica, Yersi7zia pestis or any species falling within the genera of any of the above species, including coagulase negative species of Staphylococcus. In some embodiments, the genomic library may be from an organism other than E. coli. Standard molecular biology techniques are used to generate genomic libraries from various cells or microorganisms. In one aspect, the libraries are generated and bound to nitrocellulose paper. The identified exogenous nucleic acid sequences of the present invention can then be used as probes to screen the libraries for homologous sequences.

For example, the libraries may be screened to identify homologous coding nucleic acids or homologous antisense nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213, nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of one of SEQ ID NOs. 1-6213, nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a nucleic acid complementary to one of
SEQ ID NOs. 1-6213, nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200, 300,400, or 500 consecutive nucleotides of the sequence complementary to one of SEQ ID NOs. 1- 6213, nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a nucleic acid selected from the group consisting of SEQ ID NOS.: 6214-42397, nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of one of SEQ ID NOS.: 6214-42397, nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a nucleic acid complementary to one of SEQ ID
NOS.: 6214-42397, nucleic acids comprising nucleotide sequences which hybridize under stringent conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300, 400, or 500 consecutive nucleotides of the sequence complementary to one of SEQ ID NOS.: 621442397.

The libraries may also be screened to identify homologous nucleic coding nucleic acids or homologous antisense nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213, nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of one of SEQ ID NOs. 1-6213, nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleic acid complementary to one of
SEQ ID NOs. 1-6213, nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150, 200,300,400, or 500 consecutive nucleotides of the sequence complementary to one of SEQ ID
NOs. 1-6213, nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleic acid selected from the group consisting of SEQ ID NOS.: 6214-42397, nucleic acids comprising nucleic acid sequences which hybridize under moderate conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of one of SEQ ID NOS.: 6214-42397, nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a nucleic acid complementary to one of
SEQ ID NOS.: 6214-42397 and nucleic acids comprising nucleotide sequences which hybridize under moderate conditions to a fragment comprising at least 10,15,20,25,30,35,40,50,75,100, 150,200,300,400, or 500 consecutive nucleotides of the sequence complementary to one of SEQ ID NOS.: 6214-42397.

The homologous coding nucleic acids, homologous antisense nucleic acids or homologous polypeptides identified as above can then be used as targets or tools for the identification of new, antimicrobial compounds using methods such as those described herein. In some embodiments, the homologous coding nucleic acids, homologous antisense nucleic acids, or homologous polypeptides may be used to identify compounds with activity against more than one microorganism. [Placeholder]
For example, the preceding methods may be used to isolate homologous coding nucleic acids or homologous antisense nucleic acids comprising a nucleotide sequence with at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence

selected from the group consisting of one of the sequences of SEQ ID NOS. 1- 6213, fragments comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides thereof, and the sequences complementary thereto. The preceding methods may also be used to isolate homologous coding nucleic acids or homologous antisense nucleic acids comprising a nucleotide sequence with at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleotide sequence selected from the group consisting of one of the nucleotide sequences of SEQ ID NOS.: 6214-42397, fragments comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides thereof, and the sequences complementary thereto. Identity may be measured using BLASTN version 2.0 with the default parameters. (Altschul, S. F. et al. Gapped BLAST and PSI BLAST: A New Generation of Protein Database Search Programs, Nucleic Acid Res. 25: 33893402 (1997). For example, the homologous polynucleotides may comprise a coding sequence which is a naturally occurring allelic variant of one of the coding sequences described herein. Such allelic variants may have a substitution, deletion or addition of one or more nucleotides when compared to the nucleic acids of SEQ ID NOs: 1-6213, SEQ ID NOS.: 6214-42397 or the nucleotide sequences complementary thereto.

* Additionally, the above procedures may be used to isolate homologous coding nucleic acids which encode polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid identity or similarity to a polypeptide comprising the sequence of one of SEQ ID NOs: 42398-78581 or to a polypeptpide whose expression is inhibited by a nucleic acid of one of SEQ ID NOs: 1-6213 or fragments comprising at least 5,10,15,20,25,30,35,40,50,75,100, or 150 consecutive amino acids thereof as determined using the FASTA version 3.0t78 algorithm with the default parameters.

Alternatively, protein identity or similarity may be identified using BLASTP with the default parameters, BLASTX with the default parameters, or TBLASTN with the default parameters.

(Altschul, S. F. et al. Gapped BLAST and PSI-BLAST: A New Generation of Protein Database Search Programs, Nucleic Acid Res. 25: 3389-3402 (1997).

Alternatively, homologous coding nucleic acids, homologous antisense nucleic acids or homologous polypeptides may be identified by searching a database to identify sequences having a desired level of nucleotide or amino acid sequence homology to a nucleic acid or polypeptide involved in proliferation or an antisense nucleic acid to a nucleic acid involved in microbial proliferation. A variety of such databases are available to those skilled in the art, including GenBank and GenSeq. In some embodiments, the databases are screened to identify nucleic acids with at least 97%, at least 95%, at least 90%, at least 85%, at least 80%, or at least 70% nucleotide sequence identity to a nucleic acid required for proliferation, an antisense nucleic acid which inhibits proliferation, or a portion of a nucleic acid required for proliferation or a portion of an antisense nucleic acid which inhibits proliferation. For example, homologous coding sequences may be identified by using a database to identify nucleic acids homologous to one of SEQ ID Nos.

1-6213, homologous to fragments comprising at least 10,15,20,25,30,35,40,50,75,100,150, 200,300,400, or 500 consecutive nucleotides thereof, nucleic acids homologous to one of SEQ ID NOS.: 6214-42397, homologous to fragments comprising at least 10,15,20,25,30,35,40,50,75, 100,150,200,300,400, or 500 consecutive nucleotides of one of SEQ ID NOS.: 6214-42397, nucleic acids homologous to one of SEQ ID Nos. 1-6213, homologous to fragments comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides thereof or nucleic acids homologous to the sequences complementary to any of the preceding nucleic acids.

In other embodiments, the databases are screened to identify polypeptides having at least 99%, 95%, at least 90%, at least 85%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40% or at least 25% amino acid sequence identity or similarity to a polypeptide involved in proliferation or a portion thereof. For example, the database may be screened to identify polypeptides homologous to a polypeptide comprising one of SEQ ID NOs: 42398-78581, a polypeptide whose expression is inhibited by a nucleic acid of one of SEQ ID NOs: 1-6213 or homologous to fragments comprising at least 5,10,15,20,25,30,35,40,50,75,100, or 150 consecutive amino acids of any of the preceding polypeptides. In

some embodiments, the database may be screened to identify homologous coding nucleic acids, homologous antisense nucleic acids or homologous polypeptides from cells or microorganisms other than the Escherichia coli, Staplaylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Sabnonella typhi77lurium,
Acinetobacter bauma7lnii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdo7feri, BurAcholderia cepacia, BurAcholderia fungorum, BurAcholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Co7Omebacterium diptheriae, Enterobacte7 cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria menigitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syriragae, Salmonella paratyplzi, Salmonella typhi,
Staphylcoccus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis species from which they were obtained. For example the database may be screened to identify homologous coding nucleic acids, homologous antisense nucleic acids or homologous polypeptides from microorganisms such as Acinetobacter baurnamxii, Anaplasma marginale, Aspergillus ficmigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis,
Borrella burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei,
Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata),
Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridiu7n acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidiodies immitis, Corynebacterium diptheriae, Cryptococcus neoformans,
Enterobacter cloacae, Enterococcus faeclais, Enterocccus faecium, Escherichia coli,
Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae,
Legionella pneunzophila, Listeria fnonocytogenes, Moraxella catarrhalis, Mycobacteriunn avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia
asteroides, Pastem ella haenaolytica, Pasteurella multocida, Pneu7nocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri,
Shigella sonnei Staphylococcus aureus, Staphylocccus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species, including coagulase negative Staphylococcus. In some embodiments, the homologous coding nucleic acids, homologous antisense nucleic acids, or homologous polypeptides are from an organism other than E. coli.

In another embodiment, gene expression arrays and microarrays can be employed. Gene expression arrays are high density arrays of DNA samples deposited at specific locations on a glass chip, nylon membrane, or the like. Such arrays can be used by researchers to quantify relative gene expression under different conditions. Gene expression arrays are used by researchers to help identify optimal drug targets, profile new compounds, and determine disease pathways. An example of this technology is found in U. S. Patent No. 5,807,522.

It is possible to study the expression of all genes in the genome of a particular microbial organism using a single array. For example, the arrays may consist of 12 x 24 cm nylon filters containing PCR products corresponding to ORFs from Escherichia coli, Staphylococcus aureus,
Enterococcusfaecalis, Klebsiella pnemnoniae, Pseudomonas aeruginosa, Salmonella typhimurimra,
Acinetobacter baumanza7ii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneu7noniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium lifficile, Coyynebacterium diptheriae, Enterobacter cloacae, Eyzterococcus faecium, Haemophilus influenzae, Helicobacter pylort, Legionella

pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium aviurn, Mycobacteriusn bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paf atyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus penumoniae, Streptococcus pyrogenes, Treponema pallidum, Ureaplasma urealyticum, Virio cholerae or Yersiniapestis (including the nucleic acids of SEQ ID NOs.: 6214-42397). 10 ngs of each PCR product are spotted every 1.5 mm on the filter. Single stranded labeled cDNAs are prepared for hybridization to the array (no second strand synthesis or amplification step is done) and placed in contact with the filter. Thus the labeled cDNAs are of"antisense"orientation.

Quantitative analysis is done by phosphorimager.

Hybridization of cDNA made from a sample of total cell mRNA to such an array followed by detection of binding by one or more of various techniques knwon to those in the art results in a signal at each location on the array to which cDNA hybridized. The intensity of the hybridization signal obtained at each location in the array thus reflects the amount of mRNA for that specific gene that was present in the sample. Comparing the results obtained for mRNA isolated from cells grown under different conditions thus allows for a comparison of the relative amount of expression of each individual gene during growth under the different conditions.

Gene expression arrays may be used to analyze the total mRNA expression pattern at various time points after induction of an antisense nucleic acid complementary to a proliferationrequired gene. Analysis of the expression pattern indicated by hybridization to the array provides information on other genes whose expression is influenced by antisense expression. For example, if the antisense is complementary to a gene for ribosomal protein L7/L12 in the 50S subunit, levels of other mRNAs may be observed to increase, decrease or stay the same following expression of antisense to the L7/L12 gene. If the antisense is complementary to a different 50S subunit ribosomal protein mRNA (e. g. L25), a different mRNA expression pattern may result. Thus, the mRNA expression pattern observed following expression of an antisense nucleic acid comprising a nucleotide sequence complementary to a proliferation required gene may identify other proliferation-required nucleic acids. In addition, the mRNA expression patterns observed when the bacteria are exposed to candidate drug compounds or known antibiotics may be compared to those observed with antisense nucleic acids comprising a nucleotide sequence complementary to a proliferation-required nucleic acid. If the mRNA expression pattern observed with the candidate drug compound is similar to that observed with the antisense nucleic acid, the drug compound may be a promising therapeutic candidate. Thus, the assay would be useful in assisting in the selection of promising candidate drug compounds for use in drug development.

In cases where the source of nucleic acid deposited on the array and the source of the nucleic acid being hybridized to the array are from two different cells or microorganisms, gene expression arrays can identify homologous nucleic acids in the two cells or microorganisms.

The present invention also contemplates additional methods for screening other microorganisms for proliferation-required genes. In one aspect of this embodiment, an antisense nucleic acid comprising a nucleotide sequence complementary to the proliferation-required sequences from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, BOrrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botudlinum, Clostridium difficile, Corynebacteriunz diptheriae, Enterobacter cloacae, Erlterococcus faecium, Haemophilus infl'uenzae, Helicobacter pylori, Legionella pneunaophila, Listeria rnonocytogezes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudontonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Stapliylococcus epiderinidis, Staphylococcus haemolyticus, Streptococcus mutan,s Streptococcus pneumoniae, Streptococcus pyrogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis, or a portion thereof, is transcribed in an antisense orientation in such a way

as to alter the level or activity of a nucleic acid required for proliferation of an autologous or heterologous cell or microorganism. For example, the antisense nucleic acid may be a homologous antisense nucleic acid such as an antisense nucleic acid homologous to the nucleotide sequence complementary to one of SEQ ID NOs.: 621442397, an antisense nucleic acid comprising a nucleotide sequence homologous to one of SEQ ID
Nos.: 1-6213, or an antisense nucleic acid comprising a nucleotide sequence complementary to a portion of any of the preceding nucleic acids. The cell or microorganism transcribing the homologous antisense nucleic acid may be used in a cell-based assay, such as those described herein, to identify candidate antibiotic compounds. In another embodiment, the conserved portions of nucleotide sequences identified as proliferation-required can be used to generate degenerate primers for use in the polymerase chain reaction (PCR). The PCR technique is well known in the art. The successful production of a PCR product using degenerate primers generated from the nucleotide sequences identified herein indicates the presence of a homologous gene sequence in the species being screened.

This homologous gene is then isolated, expressed, and used as a target for candidate antibiotic compounds. In another aspect of this embodiment, the homologous gene (for example a homologous coding nucleic acid) thus identified, or a portion thereof, is transcribed in an autologous cell or microorganism or in a heterologous cell or microorganism in an antisense orientation in such a way as to alter the level or activity of a homologous gene required for proliferation in the autologous or heterologous cell or microorganism. Alternatively, a homologous antisense nucleic acid may be transcribed in an autologous or heterologous cell or microorganism in such a way as to alter the level or activity of a gene product required for proliferation in the autologous or heterologous cell or microorganism.

The nucleic acids homologous to the genes required for the proliferation of Escherichia coli Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, BUrkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneuntoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter colacae, Enterococcus faecium, Haemophilus nfluenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium heprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasrraa pneun2oniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or the sequences complementary thereto may be used to identify homologous coding nucleic acids or homologous antisense nucleic acids from cells or microorganisms other than Escherichia coli, staphyloccocus aureus, Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonase aeruginosa, Salmonella typhimurium, Acinetobacter bauinaiiiiii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdoi feri,
Burkholderia cepacia, Burkholderia fungorum, BUrkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridiunz difficile, Co7ynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, My coplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Samonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis to inhibit the proliferation of cells or microorganisms other than
Escherichia coli, Stapltylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salnaonella typhimuriunz, Acinetobacter baumannii, Bacillus anthracis,
Bacteroides firagilis, Bordetella pertussis, Borrelia burgdorferi, Burlcholderia cepacia,

Burkholderia fungorum, BUrkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile,
Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, pseudomonas syringae, Salmonella paratyphi, Saln2onella typhi, Staphylococcus epidernnidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Steptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersi7tia pestis by inhibiting the activity or reducing the amount of the identified homologous coding nucleic acid or homologous polypeptide in the cell or microorganism other than Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia
burgdo7feri, BUrkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomaonas syringae, Salmonella paratyphi, salmonella typhi,
Staphylococcus epidermidis, Staphyloccoccus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or to identify compounds which inhibit the growth of cells or microorganisms other than Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannfi, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi,
Burkholderia cepacia, Bukholderia fungorum, Burlcholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faeciunz, Haenzophilus influenzae, Helicobacter pylori, Legionella pneurnophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis.
Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis as described below. For example, the nucleic acids homologous to proliferation-required genes from Escherichia coli, Staphylococcus aureus, Enterococcus faecais,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, borrelia burgdorferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallel, Campylobacter jejuni, chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Eriterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, proteus mirabilis, Pseudoiiionas putida, Pseudoiizonas syriizgae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or the sequences complementary thereto may be used to identify compounds which inhibit the growth of Acinetobacter bacumannii, Anaplasma marginale,
Aspergillus fumigatus, Bacillus anthrais, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,

Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidiolides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis,
Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, pseudomonas sy ringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shgella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnjei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticium, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia estis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the nucleic acids homologous to proliferation-required sequences from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii,
Bacillus aszthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlcholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Closridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes,
Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumo7 ? iae,
Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or
Yersinia pestis (including nucleic acids homologous to one of SEQ ID NOs.: 6214-42397) or the sequences complementary thereto (including nucleic acids homologous to one of SEQ ID NOs.: 1-
6213) are used to identify proliferation-required sequences in an organism other than E. coli.

In another embodiment of the present invention, antisense nucleic acids complementary to the sequences identified as required for proliferation or portions thereof (including antisense nucleic acids comprising a nucleotide sequence complementary to one of SEQ ID NOs.: 6214-42397 or portions thereof, such as the nucleic acids of SEQ ID NOs.: 1-6213) are transferred to vectors capable of function within a species other than the species from which the sequences were obtained. For example, the vector may be functional in Acinetobacter bacumannii, Anaplasma marginale,
Asergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,
Candida parapsilosis, Candida aguilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficuile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilius influenae,
Helicobacter pylori, Histoplasiiia capsulaturn, Klebsiella praeumoniae, Legionella pneunzoplzila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,

Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, pasteurella multiocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmo7 ? ella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidern7idis, Stap, hylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, yersinia enterocolitica,
Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the vector may be functional in an organism other than E. coli. As would be appreciated by one of ordinary skill in the art, vectors may contain certain elements that are species specific. These elements can include promoter sequences, operator sequences, repressor genes, origins of replication, ribosomal binding sequences, termination sequences, and others. To use the antisense nucleic acids, one of ordinary skill in the art would know to use standard molecular biology techniques to isolate vectors containing the sequences of interest from cultured bacterial cells, isolate and purify those sequences, and subclone those sequences into a vector adapted for use in the species of bacteria to be screened.

Vectors for a variety of other species are lmown in the art. For example, numerous vectors which function in E. coli are known in the art. Also, Pla et al. have reported an expression vector that is functional in a number of relevant hosts including : Salmonella typhimurium, Pseudomonas putida, and Pseudomonas aeruginosa. J. Bacteriol. 172 (8): 4448-55 (1990). Brunschwig and
Darzins (Gene (1992) 111: 35-4, described a shuttle expression vector for Pseudorrao7aas aeruginosa.

Vectors useful for the production of stabilized mRNA having. an increased lifetime (including antisense RNA) in Gram negative organisms are described in U. S. Provisional Patent Application
Serial Number 60/343,512, filed December 21,2001. Similarly many examples exist of expression vectors that are freely transferable among various Gram positive microorganisms. Expression vectors for Enterococcus faecalis may be engineered by incorporating suitable promoters into a pAK80 backbone (Israelsen, H., S. M. Madsen, A. Vrang, E. B. Hansen and E. Johansen. 1995.

Appl. Environ. MicrobioZ. 61: 2540-2547. A number of vectors useful for nucleic acid expression (including antisense nucleic acid expression) in Enterococcus faecalis, Staphylococcus areus as well as other Gram positive organisms are described in U. S. Patent Application Serial Number 10/032,393, filed December 21,2001.

Following the subcloning of the antisense nucleic acids complementary to proliferation required sequences from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus aiztlaracis, Bacteroides fragilis, Bordetella pertussis, Bornelia burgdorferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium
botuli7zum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus inutaiis, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, vibrio cholerae or Yers 71ia pestis or portions thereof into a vector functional in a second cell or microorganism of interest (i. e. a cell or microorganism other than the one from which the identified nucleic acids were obtained), the antisense nucleic acids are conditionally transcribed to test for bacterial growth inhibition. The nucleotide sequences of the nucleic acids irom Escherichia coli,
Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa,

Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis,
Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacteriu771 leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae,
Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus nautans, Streptococcus pneurnoniae, Streptococcus pyogenes,
Treporzema pallidurn, Ureaplasma
urealyticu7n, Vibrio cholerae or Yersinia pestis that, when transcribed, inhibit growth of the second cell or microorganism are compared to the grown genomic sequence of the second cell or microorganism to identify the homologous gene from the second organism. If the homologous sequence from the second cell or microorganism is not known, it may be identified and isolated by hybridization to the proliferation-required Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi,
Burlcholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Coynebacteriufn diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacer pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalix, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrlaoeae, Neissenia me7aingitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptoccus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis sequence of interest or by amplification using PCR primers based on the proliferation-required nucleotide sequence of interest as described above. In this way, sequences which may be required for the proliferation of the second cell or microorganism may be identified.

For example, the second microorganism may be Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,
Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida lsefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium,
Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis,
Nocardia asteroides, Pasteurella haemolytica, Pasteurella rnultocida, Pneuniocystis carinii, Proteus nzirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidersnidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma
urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,

Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the second microorganism is an organism other than E. coli.

The homologous nucleic acid sequences from the second cell or microorganism which are identified as described above may then be operably linlced to a promoter, such as an inducible promoter, in an antisense orientation and introduced into the second cell or microorganism. The techniques described herein for identifying Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthnacis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlcliolderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytoge7es, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema palidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis genes required for proliferation may thus be employed to determine whether the identified nucleotide sequences from a second cell or microorganism inhibit the proliferation of the second cell or microorganism. For example, the second microorganism may be
Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlcholderia cepacia,
Burlcholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida Arrusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridiuin botulinuin, Clostridiuiii difficile, Clostridium perfriiigens, Coccidioides iinmitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haernophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumo7tiae, Legionel1a pneumophila, Listeria
Histoplasma capsulatum, jKlebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocar dia asteroides, Pasteurella haemolytica, Pasteurella multocida, pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudornonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus nautans, Streptococcus pyogenes, Treponeena pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the second microorganism may be an organism other than E. coli.

Antisense nucleic acids required for the proliferation of microorganisms other than
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebisella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,

Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile,
Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or the genes corresponding thereto, may also be hybridized to a microarray containing the Escherichia coli,
Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa,
Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis,
Bordetella pertusis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzze, Helicobacter pylori, Legioizella piieuinophila, Listeria monocytogeiies, Moraxella catarrlzalis, Mycobacteriuni avium, Mycobacterium bovis, Mycobacteriuna leprae, Mycobacteriurra tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidernaidis, Staphylococcus haenaolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the nucleic acids of SEQ ID NOs.: 621442397) to gauge the homology between the Eselaerichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Bunleholdenia cepacia, Burlzholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faeciuna, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella cata7rhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, pasteurella multocida, Proteus mirabilis, Pseudomonas putida, pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis sequences and the proliferation-required nucleic acids from other cells or microorganisms. For example, the proliferation-required nucleic acid may be from
Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium diffcile, Clostridium perfringens, Coccidioddes immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis,

Nocardia asteroides, Pasteurella haemolytica, Pasteurella n2ultocida, Pneunaocystis carinii, Proteus rnirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmotiella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the proliferation-required nucleotide sequences from
Escherichia coli, Staphlococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acnetobacter baumannii, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile,
Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus
iiiflueizzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or homologous nucleic acids are used to identify proliferation-required sequences in an organism other than E. coli.

In some embodiments of the present invention, the proliferation-required sequences may be from an organism other than E. coli. The proliferation-required nucleic acids from a cell or microorganism other than Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis,
Bacteroids fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, campylobacte rjejuni, Chlamydia pneumoniae,
Chlamydia trac7aomatis, Clostridium acetobutylicuna, Clostridiuyra botulifium, Clostnidiurn difficile,
Corynebacterium diptlaeriae, Efaterobacter cloacae, Enterococcus faeciufra, Haemophilus influenzae, Helicobacter pylori, Legioiiella pneuniophila, Listeria moiiocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasnaa genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella wultocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyoge7ses,
Treponema pallidum, Ureaplasma urealyticu7n, Vibrio cholerae or Yersinia pestis may be hybridized to the array under a variety of conditions which permit hybridization to occur when the probe has different levels of homology to the nucleotide sequence on the microarray. This would provide an indication of homology across the cells or microorganisms as well as clues to other possible essential genes in these cells or microorganisms.

In some embodiments of the present invention, the essential gene products described herein are used in methods of identifying a target on which a compound that inhibits cellular proliferation acts. Such methods are described in the U. S. Patent Application entitled METHODS FOR
IDENTIFYING THE TARGET OF A COMPOUND WHICH INHIBITS CELLULAR
PROLIFERATION, filed February 8,2002. As employed herein, some embodiments of methods used to identify a target on which a compound that inhibits cellular proliferation acts utilize collections or cultures of strains comprising strains which either overexpress a different gene product which is required for cellular proliferation (such as the gene products described herein) or underexpress a different gene product (such as the. gene products described herein) which is required for cellular proliferation (i. e. at least some of the strains in the culture overexpress or underexpress a gene product required for cellular

proliferation). In some embodiments, the present invention uses collections or cultures of strains comprising both strains which overexpress gene products required for cellular proliferation and strains which underexpress the same gene products required for cellular proliferation. Preferably, each of the strains present in the culture or collection either overexpresses or underexpresses a different gene product which is required for cellular proliferation (i. e. all of the strains in the culture overexpress or underexpress a gene product required for cellular proliferation). However, in some embodiments, the culture or collection may include one or more strains which do not overexpress or underexpress a gene product which is required for proliferation. The gene product which is overexpressed or underexpressed in each strain may be any gene product which is required for cellular prolifereation, including a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 621442397, a gene product comprising an amino acid sequence selected from the group consisting of
SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

As used herein the term"culture"refers to a plurality of strains growing in a single aliquot of a liquid growth medium and the term"collection"refers to a plurality of strains each of which is growing in a separate aliquot of liquid growth medium or a different location on a solid growth medium.

In some embodiments, if desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product described herein which is required for cellular proliferation. In this embodiment, the gene products which are overexpressed or underexpressed in one or more of the strains may be functionally related or functionally unrelated. This may facilitate the identification of compounds when two or more gene products share similar functions in the cell or where the cell has multiple biochemical pathways which lead to a particular end product.

Alternatively, if the gene product described herein to be overexpressed or underexpressed is encoded by a gene which is part of an operon containing a plurality of genes, the desired gene may be overexpressed or underexpressed while the remaining genes in the operon are expressed at levels where they do not impact the ability of the cell to grow in the presence of a particular compound.

For example, the desired gene may be placed under the control of a regulatable promoter, a transcriptional terminator may be placed 3'of the desired gene and a promoter, preferably a constitutive promoter, may be placed 3'of the transcriptional terminator and 5'of the remaining genes in the operon.

In some embodiments, the culture or collection of strains may comprise a strain which overexpresses or underexpresses a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213.

In some embodiments, the culture or collection of strains may comprise strains which in aggregate overexpress or underexpress at least two gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 10 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID
NOS.: 1-6213, at least 20 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 30 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.:
1-6213, at least 50 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 100 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, at least 300 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213 or more than 300 gene products whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOS.: 1-6213, wherein each strain in the culture or collection of strains overexpresses or underexpresses a single gene product whose activity or level is inhibited by a nucleic acid selected from the group consisting of
SEQ ID NOs. 1-6213. Alternatively, if desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product whose activity or level is inhibited by a nucleic acid selected from the group consisting of SEQ ID NOs. 1-6213.

In other embodiments, the culture or collection of strains may comprise a strain which overexpresses or underexpresses a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397. In some embodiments, the culture or collection of strains may comprise strains which in aggregate overexpress or underexpress at least two gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 10 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 20 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 30 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 50 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 621442397, at least 100 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, at least 300 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397 or more than 300 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 6214-42397, wherein each strain in the culture or collection of strains overexpresses or underexpresses a single gene product encoded by a nucleic acid selected from the group consisting of SEQ ID NOs. 6214-42397.

Alternatively, if desired, one or more strains in the culture or collection of strains may overexpress or underexpress more than one gene product encoded by a nucleic acid selected from the group consisting of SEQ ID NOs. 6214-42397.

In some embodiments the culture or collection of strains comprises a strain in which a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 is overexpressed or underexpressed. In some embodiments, the culture or collection of strains may comprise strains which in aggregate overexpress or underexpress at least two gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 10 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 20 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 30 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 50 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 100 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581, at least 300 gene products comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42938-78581 or more than 300 gene products comprising an amino acid sequence selected from the group consisting of SEQID NOs.: 42938-78581, wherein each strain in the culture or collection of strains overexpresses or underexpresses a single gene product selected from the group consisting of SEQ ID NOs. 42938-78581. Alternatively, if desired one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product selected from the group consisting of SEQ ID NOs. 42938-78581.

In other embodiments, the culture or collection of strains comprises a strain in which at least one of the gene products encoded by a homologous coding nucleic acid as defined above is overexpressed or underexpressed. In some embodiments, the culture or collection of strains may comprise strains which in aggregate overexpress or underexpress at least 2, at least 10, at least 20, at least 30, at least 50, at least 100, at least 300 or more than 300 gene products encoded by a homologous coding nucleic acid as defined above. If desired the culture or collection of strains may comprise one or more strains which overexpress or underexpress more than one gene product encoded by a homologous coding nucleic acid. In further embodiments, the culture or collection of strains comprises a strain in which at least one, at least 10, at least 20, at least 30, at least 50, at least
100, at least 300 or more than 300 homologous polypeptides as defined above is overexpressed or underexpressed. If desired the culture or collection of strains may comprise one or more strains which overexpress or underexpress more than one homologous polypeptide.

For example, in some embodiments, the culture or collection of strains comprises a strain in which at least one gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using
BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as

determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product. In some embodiments, the culture or collection of strains may comprise strains in which in aggregate at least 2, at least 10, at least 20, at least 30, at least 50, at least 100, at least 300, or more than 300 gene products selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of
SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213 is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product.

If desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product selected from the group consisting of a gene product having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleic acid encoding a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213, a gene product having at least 25% amino acid identity as determined using FASTA version 3.0t78 with the default parameters to a gene product whose expression is inhibited by an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under stringent conditions, a gene product encoded by a nucleic acid which hybridizes to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 under moderate conditions, and a gene product whose activity may be complemented by the gene product whose activity is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs: 1-6213.

In further embodiments, the culture or collection of strains comprises a strain in when au least one gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product. In some embodiments, the culture or collection of strains comprises a strain or a group of strains in which in aggregate at least 2, at least 10, at least 20, at least 30, at least 50, at least 100, at least 300, or more than 300 gene products encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version

2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product.

If desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of a nucleic acid comprising a nucleic acid having at least 70% nucleotide sequence identity as determined using BLASTN version 2.0 with the default parameters to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 621442397, a nucleic acid comprising a nucleotide sequence which hybridizes to a sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under stringent conditions, and a nucleic acid comprising a nucleotide sequence which hybridizes to a nucleotide sequence selected from the group consisting of SEQ ID NOS.: 6214-42397 under moderate conditions.

In additional embodiments, the culture or collection of strains comprises a strain in which at least one gene product comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ
ID NOs: 42938-78581 is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product. In some embodiments, the culture or collection of strains comprises a strain or a group of strains in which in aggregate at least 2, at least 10, at least 20, at least 30, at least 50, at least 100, at least 300, or more than 300 gene products comprising a polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ ID NOs: 42938-78581 is overexpressed or underexpressed, wherein each strain overexpresses or underexpresses one gene product.

If desired, one or more of the strains in the culture or collection of strains may overexpress or underexpress more than one polypeptide selected from the group consisting of a polypeptide having at least 25% amino acid identity as determined using FASTA version 3.0t78 to a polypeptide selected from the group consisting of SEQ ID NOs.: 42938-78581 and a polypeptide whose activity may be complemented by a polypeptide selected from the group consisting of SEQ
ID NOs: 42938-78581.

The methods of the present invention may be used to identify the targets of compounds which inhibit the proliferation of any desired cell or organism. In some embodiments, these methods are employed to identify the targets of compounds which inhibit the proliferation of bacteria, fungi, or protozoans. In further embodiments, these methods are employed to identify the targets of compounds which inhibit the growth of an organism selected from the group consisting of
Acinetobacter baumannii, Anaplma marginale, Aspergillus fumigatus, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridiui7i botulinuin, Clostridium difficile, Clostridium peifriizgens, Coccidioides inzmitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacteriusn tuberculosis, Mycoplasrna genitalium, Mycoplasrna pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis,

Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species.

Overexpression may be obtained using a variety of techniques familiar to those skilled in the art. For example, overexpression may be obtained by operably linking a gene encoding a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 621442397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, or a gene product comprising a homologous polypeptide to a promoter which transcribes a higher level of mRNA encoding or comprising the gene product than does a wild type cell.

A variety of promoters may be used to overexpress the gene product described herein, including a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide. The promoters used to overexpress the gene product may be relatively strong promoters, promoters which possess a moderate level of activity, or relatively weak promoters and may be either constitutive or regulatable promoters. In some embodiments, several strains, each of which overexpresses the gene product to a different extent, may be used in order to optimize the degree of overexpression of the gene product.

In some embodiments, each of the gene products required for proliferation may be placed under the control of several different promoters of varying strengths to create several different strains which express the gene product at varying levels. The level of expression of the gene product in each of the strains is compared to that in wild type cells in order to identify a promoter which provides a desired level of expression relative to wild type cells (i. e. a desired level of overexpression or underexpression). The strain having the desired level of expression is then included in a culture or collection of strains to be contacted with a test compound as discussed below. Examples of suites of regulatable promoters having varying strengths that are useful for the expression of gene products at varying levels are described in U. S. Patent Application Serial Number 10/032,393, filed on December 21,2002.

The promoter is selected to be active in the type of cell in which the gene product is to be expressed. For example, for overexpression of the gene product in mammalian cells, the gene encoding the gene product may be operably linked to promoters such as the SV40 promoter, the metallothionine promoter, the MMTV promoter, the RSV promoter, the tetP promoter, the adenovirus major late promoter or other promoters known to those skilled in the art. In yeast, the gene encoding the gene product may be operably linked to promoters such as the CYC1, ADHI, ADHII, GAL1, GAL10, PHO5, PGK or other promoters used in the art. Similarly, in bactena, me gene encoding the gene product may be operably linlced to the, SP6, T3, trc promoter, lac promoter, temperature regulated lambda promoters, the Bacillus aprE and nprE promoters (U. S.

Patent No. 5,387,521), the bacteriophage lambda PL and PR promoters (Renaut, et al., (1981) Gene 15: 81) the trp promoter (Russell, et al., (1982) Gene 20: 23), the tac promoter (de Boer et al., (1983) Proc. Natl. Acad. Sci. USA 80: 21), B. subtilis alkaline protease promoter (Stahl et al, (1984) J. Bacteriol. 158,411-418) alpha amylase promoter of B. subtilis (Yang et al., (1983) Nucleic Acids Res. 11,237-249) or B. amyloliquefaciens (Tarlcinen, et al, (1983) J. Biol. Chem.

258,1007-1013), the neutral protease promoter from B. subtilis (Yang et al, (1984) J. Bacteriol.

160,15-21), T7 RNA polymerase promoter (Studier and Moffatt (1986) J Mol Biol. 189 (1) : 11330), B. subtilis xyl promoter or mutant tetR promoter active in bacilli (Geissendorfer & Hillen (1990) Appl. Microbiol. Biotechnol. 33: 657-663), Staphylococcal enterotoxin D promoter (Zhang and Stewart (2000) J. Bacteriol. 182 (8): 2321-5), cap8 operon promoter from Staphylococcus aureus (Ouyang et al., (1999) J. Bacteriol. 181 (8): 2492-500), the lactococcal nisA promoter (Eichenbaum (1998) Appl Environ Microbiol. 64 (8): 2763-9), promoters from in Acholeplasma laidlawEi (Jarhede et al., (1995) Microbiology 141 (Pt 9): 2071-9), porA promoter of Neissenia menin? gitidis (Sawaya et al., (1999) Gene 233: 49-57), the fbpA promoter of Neisseria gonorrhoeae (Forng et al., (1997) J.

Bacteriol. 179: 3047-3052), Coiyiiebacteriuin diphtheriae toxin gene promoter (Schmitt and Holmes (1994) J. Bacteriol. 176 (4): 1141-9), the hasA operon promoter from Group A Streptococci (Alberti et al., (1998) Mol Microbiol 28 (2): 343-53), the rpoS promoter of Pseudomonas putida (IZojic and Venturi (2001) J. Bacteriol. 183: 3712-3720), the Acis1etobacter baurnaranii phosphate regulated ppk gene promoter (Gavigan et al., Microbiology 145: 2931-7 (1999)); the Acinetobacter baufnarznii adhC1 promoter which is induced under iron limitation and repressed when the cells are cultured in the presence of free inorganic iron (Echenique et al., Microbiology 147: 2805-15 (2001)); the flaB promoter ofpGK12 active in Borrelia burgdorferi (Sartakova et al., Proc Natl Acad Sci U S A.

97 (9): 4850-5 (2000)); the use of Ptrc promoter results in strong inducer-dependent expression in Burlzlaolderia spp (Santos et al., FEMS Microbiol Lett 195 (1) : 91-6 (2001)); the iron regulated sodA promoter of Bordetella pertussis (Graeff-Wohlleben et al., J Bacteriol 179 (7): 2194-201 (1997)); UV-inducible bon and uviAB promoters in Clostrdia spp (Garnier and Cole Mol Microbiol 2 (5): 607-14 (1988)); the heat-inducible clpB promoter of Campylobacter jejuni (Thies et al., Gene 230 (1) : 61-7 (1999)); promoters carrying bacteriophage C1 operator sites in Klebsiella pneumoniae (Schoefield et al, J Bacteriol 183 (23): 6947-50 (2001)); the Proteus nzirabilis ureR promoter (Poore et al., J Bacteriol 183 (15): 4526-35 (2001)); and the heat-inducible groESL promoter in Listeria monocytogefaes, and the IPTG inducible promoter in pLEX5BA (Krause et al., J. Mol. Biol. 274: 365 (1997). In another embodiment, which may be useful in Staphylococcus aureus, the promoter is a novel inducible promoter system, XylT5, comprising a modified T5 promoter fused to the oxyl0 operator from the xylA promoter of Staphylococcus aureus. This promoter is described in U. S.

Patent Application Serial Number 10/032,393. In another embodiment the promoter may be a two component inducible promoter system in which the T7 RNA polymerase gene is mtegratea on Ine chromosome and is regulated by lacUV5 lac0 (Brunschwig, E. and Darzins, A. 1992. Gene 111: 35-41, and a T7 gene 10 promoter, which is transcribed by T7 RNA polymerase, is fused with a lac0 operator. In another embodiment the promoter may be the promoters from the plasmids pEPEF3 or pEPEF14, which harbor xylose inducible promoters functional in E. faecalis, described in U. S. Patent Application Serial No. 10/032,393. Other promoters which may be used are familiar to those skilled in the art. In fungi, the gene encoding the gene product may be operably linked to the CaACTl promoter (Morschhauser, Mol. Gen. Genet. 257: 412-420 (1998), or other promoters familiar to those skilled in the art. It will appreciated that other combinations of organisms and promoters may also be used in the present invention.

In some embodiments, overexpression may be achieved by using homologous recombination to replace the natural promoter which drives expression of the proliferation-required genes described herein with a regulatable promoter. For example, the methods described in U. S.

Patent Application 09/948,993 may be used to place the gene required for proliferation under the control of a regulatable promoter. Examples of gene products, which are encoded by genes that can be overexpressed by regulatable promoters introduced by such promoter replacement methods include a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs. : 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

Briefly, in some embodiments of these methods in which natural promoters are replaced by regulatable promoters, the cells may be haploid, such as bacterial cells. Regulatable promoters that are useful for promoter replacement in bacterial cells include, but are not limited to, the promoters described in U. S. Patent Application Serial Number 10/032,393 filed December 21,2001. A linear promoter replacement cassette comprising a regulatable promoter flanked by nucleotide sequences having homology to the natural promoter is introduced into the cell. In some embodiments, the cassette also comprises a nucleotide sequence encoding a selectable marker or a marker whose expression is readily identified. The cassette may be a double stranded nucleic acid or a single stranded nucleic acid as described in U. S. Patent Application Serial Number 09/948,993. Upon homologous recombination, the natural promoter is replaced with the regulatable promoter, leaving the gene required for proliferation under the control of the regulatable promoter. Strains in which the gene required for proliferation is under control of the regulatable promoter are grown under conditions in which the regulatable promoter provides a level of the proliferation-required gene product which is above the level in a wild type cell. For example, the strains may be grown in the presence of an inducer which induces expression from the regulatable promoter, or under conditions in which the action of a repressor on the regulatable promoter is reduced or eliminated.

Alternatively, rather than replacing the native promoters of each of the genes encoding a proliferation-required gene product described herein with a single desired replacement promoter, a plurality of replacement promoters which provide desired expression levels for the gene products to be overexpressed or underexpressed are used. The method is performed as described above except that rather than using a single labeled primer complementary to a nucleotide sequence within the single replacement promoter, a plurality of labeled primers complementary to suitable nucleotide sequences in the plurality of replacement promoters are used.

Alternatively, in embodiments in which the level or activity of proliferation-required gene products described herein is reduced by transcribing an antisense nucleic acid complementary to at least a portion of the genes encoding such gene products, the strains may be designed such that the length of the nucleotide sequence encoding the antisense nucleic acid is different for each gene.

Amplification reactions are performed as described above using primers at each end of the gene encoding the antisense nucleic acid such that the amplification product corresponding to each gene has a unique length or a dye which allows it to be distinguished from other amplification products of the same length. Alternatively, the lengths of the nucleotide sequences encoding the antisense nucleic acids may not be unique for each gene, but the primers used in the amplification reaction may be selected such that the length of the amplification product corresponding to each gene is unique.

In another embodiment, the native promoters may be replaced with promoters which include therein or adjacent thereto a unique nucleotide sequence which is distinct from that present in the other replacement promoters in the strains in the culture or collection of strains. In this embodiment, each promoter includes or has adjacent thereto a unique"tag"which may be used to identify strains which proliferate more rapidly or more slowly in the culture or collection of strains.

The tag may be detected using hybridization based methods or amplification based methods, including the amplification method which generates amplification products having a unique size for each proliferation required gene described above.

Alternatively, the native promoter which directs the transcription of the proliferationrequired genes described herein may rendered regulatable by inserting a regulatory element into the chromosome of the cell via homologous recombination such that the regulatory element regulates the level of transcription from the promoter. Examples of gene products, which are encoded by genes that have promoters which can be rendered regulatable by regulatory elements inserted by such methods include a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

A variety of regulatory elements may be used to regulate the expression of essential gene products described herein. The regulatory element may be an operator which is recognized by a repressor (e. g. lac, tet, araBAD repressors) or a nucleotide sequence which is recognized by a transcriptional activator. In some embodiments, the regulatory element may be a transcriptional terminator, a nucleotide sequence which introduces a bend in the DNA or an upstream activating sequence. A linear regulatory element insertion cassette comprising a regulatory element flanked by nucleotide sequences having homology to the natural promoter is introduced into the cell. In some embodiments, the cassette also comprises a nucleotide sequence encoding a selectable marker or a marker whose expression is readily identified. The cassette may be a double stranded nucleic acid or a single stranded nucleic acid as described in U. S. Patent Application Serial Number 09/948,993. Upon homologous recombination, the regulatory element is inserted into the chromosome, leaving the gene required for proliferation under the control of the regulatory element. Strains in which the gene required for proliferation is under control of the regulatory element are grown under conditions in which the regulatable promoter provides a level of the proliferation-required gene product which is above the level in a wild type cell. For example, the strains may be grown in the presence of an inducer which induces expression from the promoter, or under conditions in which the action of a repressor on the promoter is reduced or eliminated. It will be appreciated that the amplification method which generates amplification products having a unique size for each proliferation required gene may be used to detect strains which are overrepresented or underrepresented in the culture or collection of strains. For example, if desired, primers complementary to a nucleotide sequence within the regulatory element may be used in the amplification reaction.

The promoter replacement cassette or regulatory element insertion cassette may be a double stranded nucleic acid, such as an amplicon generated through PCR or other amplification methods, or a single stranded nucleic acid, such as an oligonucleotide. For example, single stranded nucleic acids may be introduced into the chromosome using the methods described in Ellis et al., PNAS 98: 6742-6746,2001.

In some embodiments, the cell into which the promoter replacement cassette or regulatory element insertion cassette is introduced has an enhanced frequency of recombination. For example, the cells may lack or have a reduced level or activity of one or more exonucleases which would ordinarily degrade the DNA to be inserted into the chromosome. In further embodiments, the cells may both lack or have reduced levels of exonucleases and express or overexpress proteins involved in mediating homologous recombination. For example, if the methods are performed in Esche7ichia coli or other enteric prokaryotes, cells in which the activity of exonuclease V of the
RecBCD recombination pathway, which degrades linear nucleic acids, has been reduced or eliminated, such as recB, recC, or recD mutants may be used. In some embodiments, the cells have mutations in more than one of the recB, recC, and recD genes which enhance the frequency of homologous recombination. For example the cells may have mutations in both the recB and recC genes.

The promoter replacement or regulatory element insertion methods may also be performed in Eschericlaia coli cells in which the activity of the RecET recombinase system of the Rac prophage has been activated, such as cells which carry an sbcA mutation. The RecE gene of the rac prophage encodes ExoVIII a 5'-3'exonuclease, while the RecT gene of the Rac prophage encodes a single stranded DNA binding protein which facilitates renaturation and D-loop formation. Thus, the gene products of the RecE and RecT genes or proteins with analogous functions facilitate homologous recombination. The RecE and RecT genes lie in the same operon but are normally not expressed. However, sbcA mutants activate the expression the RecE and RecT genes. In some embodiments, the methods may be performed in cells which carry mutations in the recB and recC genes as well as the sbcA mutation. The RecE and RecT gene may be constitutively or conditionally expressed. For example, the methods may be performed in E. coli strain JC8679, which carries the sbcA23, recB21 and recC22 mutations.

In some embodiments, the methods may be performed in Escherichia coli cells in which recombination via the RecF pathway has been enhanced, such as cells which carry an sbcB mutation.

It will be appreciated that the RecE and RecT gene products, or proteins with analogous functions may be conditionally or constitutively expressed in prokaryotic organisms other than E. coli. In some embodiments, these proteins may be conditionally or constitutively expressed in
Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burlcholderia cepacia,

Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida kusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faeciurn, Eschericlaia coli, Haenaophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typlli Salmonella typhirnurium, Shigella boydii, Slaigella dysenteriae, Sliigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptocöccus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica,
Yersinia pestis or any species falling within the genera of any of the above species. For example, plasmids encoding these gene products may be introduced into the organism. If desired, the coding sequences encoding these gene products may be optimized to reflect the codon preferences of the organism in which they are to be expressed. Similarly, in some embodiments, the organism may contain mutations analogous to the recB, recC, recD, sbcA or sbcB mutations which enhance the frequency of homologous recombination.

In further embodiments, the promoter replacement or regulatory element insertion methods may be conducted in cells which utilize the Red system of bacteriophage lambda (X) or analogous systems from other phages to enhance the frequency of homologous recombination. The Red system contains three genes, (y, P and exo whose products are the Gam, Bet and Exo proteins (see
Ellis et al. PNAS 98: 6742-6746,2001. The Gam protein inhibits the RecBCD exonuclease V, thus
pennitting Beta and Exo to gain access to the ends of the DNA to be integrated and facilitating homologous recombination. The Beta protein is a single stranded DNA binding protein that promotes the annealing of a single stranded nucleic acid to a complementary single stranded nucleic acid and mediates strand exchange. The Exo protein is a double-stranded DNA dependent 5'-3' exonuclease that leaves 3'overhangs that can act as substrates for recombination. Thus, constitutive or conditional expression of the X Red proteins or proteins having analogous functions facilitates homologous recombination.

It will be appreciated that the X Beta, Gam and Exo proteins, or proteins with analagous functions may be expressed constitutively or conditionally in prokaryotic organisms other than E. coli. In some embodiments, these proteins may be conditionally or constitutively expressed in
Acinetobacte baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Boorelia burgdorferi, Burlclaolderia cepacia,
Burkholderia funogrum, Burlzlaolderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumo7liae, Chlamydia trachomatis, Clostridium acetobutylicu7n,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faeciurn, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneutnoniae, Legionella pnez?mophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasrraa pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Prolteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongor@,

Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. For example, plasmids encoding these gene products may be introduced into the organism. If desired, the coding sequences encoding these gene products may be optimized to reflect the codon preferences of the organism in which they are to be expressed.

In some embodiments, the cells may have an increased frequency of homologous recombination as a result of more than one of the aforementioned characteristics. In some embodiments, the enhanced frequency of recombination may be a conditional characteristic of the cells which depends on the culture conditions in which the cells are grown. For example, in some embodiments, expression of the X Red Gam, Exo, and Beta proteins or recE and recT proteins may be regulated. Thus, the cells may have an increased frequency of homologous recombination as a result of any combination of the aforementioned characteristics. For example, in some embodiments, the cell may carry the sbcA and recBC mutations.

In some embodiments, a linear double stranded DNA to be inserted into the chromosome of the organism is introduced into an organism constitutively or conditionally expressing the recE and recT or the X Beta, Gam and Exo proteins or proteins with analogous functions as described above.

In some embodiments, the organism may be Acinetobacter baumannii, Anaplasma marginale,
Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Camplylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,
Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides in77nitis, Co7ynebacteriunl diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae,
Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacteirum avium, Mycobacterium bovis,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas puticla, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmo71ella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus fnutans, Streptococcus pyogenes, Treponerna pallidum, Ureaplasrna urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments, the double stranded DNA may be introduced into an organism having the recBC and sbcA mutations or analogous mutations.

In other embodiments, a single stranded DNA to be inserted into the chromosome of the organism is introduced into an organism expressing the ! Beta protein or a protein with an analogous function. In some embodiments the single stranded DNA is introduced into an organism expressing both the X Beta and Gam proteins or proteins with analogous functions. In further embodiments, the single stranded DNA is introduced into an organism expressing the k Beta, Gam and Exo proteins or proteins with analogous functions. The X proteins or analogous proteins may be expressed constitutively or conditionally. In some embodiments, the organism may be
Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdolfel-i, Bul-Icliolderia cepacia,

Burkholderia fungorum, Burkkholderia mallei, Campylbacter jejuni, Candida albicans, candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridum perfringens, Coccidioides immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarr1zalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudoinoizas putida, Pseudoinoiias syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Slaigella sonnei,
Staplzylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species.

In some embodiments, the linear nucleic acid may be introduced into the chromosome of a first organism which has an enhanced frequency of homologous recombination and then transferred to a second organism which is less amenable to direct application of the present methods. For example, the linear nucleic acid may be introduced into the chromosome of E. coli and transferred into a second organism via conjugation or transduction. After introduction into the second organism, the nucleic acid is inserted into the chromosome of the second organism via homologous recombination, thereby effectively transferring the regulatory element from the chromosome of the first organism into the corresponding location in the chromosome of the second organism.

In other embodiments, the cells may be diploid cells, such as fungal cells. In some embodiments, one copy of the gene encoding the proliferation-required gene product may be disrupted, rendering it inactive. In further embodiments, one copy of the gene encoding the proliferation-required gene product may be disrupted and the other copy of the gene encoding the proliferation-required gene product may be placed under the control of a regulatable promoter.

Such strains may be generated by disrupting the first copy of the gene encoding the proliferationrequired gene product by homologous recombination using a disruption cassette comprising a nucleotide sequence encoding an expressible dominant selectable marker flanked on each side by nucleic acids homologous to the target sequence to be disrupted. The second copy of the gene encoding the proliferation-required gene product may be placed under the control of a regulatable promoter by homologous recombination using a promoter replacement cassette comprising a regulatable promoter flanked on each side by nucleic acids homologous to the natural promoter for the proliferation-required gene. The promoter replacement cassette may also include a nucleotide sequence encoding a selectable marker located 5'of the regulatable promoter but between the nucleic acids homologous to the natural promoter.

In other embodiments, overexpression may be achieved by operably linking a proliferationrequired gene product described herein to a desired promoter in a vector. The vector may be a vector which replicates extrachromosomally or a vector which integrates into the chromosome. For example, if the vector is to be used in bacterial cells, the vector may be a pBR322 based vector or a bacteriophage based vector such as P1 or lambda. If the vector is to be used in Saccharornyces cerevisae, it may be a vector based on the 2 micron circle or a vector incorporating a yeast chromosomal origin of replication. If the vector is to be used in mammalian cells, it may be a retroviral vector, SV40 based vector, a vector based on bovine papilloma virus, a vector based on adenovirus, or a vector based on adeno-associated virus. If the vector is to be used in Candida albicans it may be a vector comprising a promoter selected from the group consisting of the CaPCKl, MET25, MAL2, PH05, GAL1, 10, STE2 or STE3 promoters. In some embodiments, the vectors described in the following publications may be used: CIplO, an efficient and convenient integrating vector for Candida albicans. Murad et al., Yeast 16 (4): 325-7 (2000); Transforming vector pCPW7, Kvaal et al.,; Infect Immun 67 (12): 6652-62 (1999); Transforming vector pCWOP16, Kvaal et al.,: Infect Immun 65 (11): 4668-75 (1997); double-ARS vector, pRMI, to be used for direct cloning in Ca by complementation of the histidine auxotrophy of strain CA9, Pla et al., Gene 165 (1) : 115-20

(1995); pMK16, that was developed for the transformation of C. albicans and carries an ADE2 gene marker and a Candida autonomously replicating sequence (CARS) element promoting autonomous replication (cited in Sanglard and Fiechter Yeast 8 (12): 1065-75 (1992); A plasmid vector (denoted pRC2312) was constructed, which replicates autonomously in Escherichia coli, Sacclzaroonyces cerevisiae and Candida albicans. It contains LEU2, URA3 and an autonomously replicating sequence (ARS) from C. albicans, Cannon et al., Mol Gen Genet 235 (23): 453-7 (1992); Expression vector (CIplO-MAL2p) for use in Candida albicans has been constructed in which a gene of interest can be placed under the control of the CaMAL2 maltase promoter and stably integrated at the CaRP10 locus (Backen et al., Yeast 16 (12): 1121-9 (2000)); (Vollcer, R. S., A. Sonneborn, C. E. Leuker, and J. F. Ernst. 1997. Efglp, an essential regulator of morphogenesis of the human pathogen Candida albicans, is a member of a conserved class of bHLH proteins regulating morphogenetic processes in fungi. EMBO 16: 1982-1991.); and a C. albicans transformation vector containing the C. albicans URA3 gene, a Candida ARS sequence, and a portion of the Saccharomyces cerevisiae 2 microns circle containing the replication origin was constructed. Goshorn et al., Infect Immun 60 (3): 876-84 (1992). A variety of other vectors suitable for use in foregoing organisms or in any other organism in which the present invention is to be practiced are familiar to those skilled in the art.

Underexpression of a proliferation-required gene product described herein may be obtained in a variety of ways. For example, in one embodiment underexpression of the proliferationrequired gene product may be achieved by providing an agent, such as an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, an antisense nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a nucleic acid complementary to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 4239878581, a nucleic acid complementary to a nucleic acid which encodes at least 5,10,15,20,25,30, 35,40,50,75,100, or 150 consecutive amino acids of a polypeptide sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a homologous antisense nucleic acid, an antisense nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of a homologous nucleic acid, a nucleic acid complementary to a homologous coding nucleic acid, a nucleic acid complementary to at least 10,15,20,25,30,35,40, 50,75,100,150,200,300,400, or 500 consecutive nucleotides of a homologous coding nucleic acid, a nucleic acid complementary to a nucleic acid which encodes a homologous polypeptide, or a nucleic acid complementary to a nucleic acid which encodes at least 5, 10,15,20,25,30,35,40,50, 75,100, or 150 consecutive amino acids of a homologous polypeptide, which reduces the level or activity of the gene product within the cell. In one embodiment, the agent may comprise an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213 which is complementary to a nucleic acid encoding the proliferation-required gene product or complementary to a portion of a nucleic acid encoding the proliferation-required gene product.

In one example of antisense-inhibition-based underexpression, a nucleic acid which encodes the antisense nucleic acid may be operably linked to a regulatable promoter. When grown under appropriate conditions, such as media containing an inducer of transcription or an agent which alleviates repression of transcription, the antisense nucleic acid is expressed in the cell, thereby reducing the level or activity of the gene product within the cell. In some embodiments, the concentration of the inducer of transcription or the agent which alleviates repression of transcription may be varied to provide optimal results. Such methods have been described previously herein and in U. S. Patent Application Serial Number 09/815,242, U. S. Patent Application Serial Number 09/492,709, U. S. Patent Application Serial Number 09/711,164, or U. S. Patent Application Serial Number 09/741, 669.

Alternatively, underexpression of a proliferation-required gene product described herein may be achieved by constructing strains in which the expression of the gene product is under the control of a constitutive or regulatable promoter using methods such as those described above with respect to methods in which the gene product is overexpressed. To provide cells which underexpress the gene product, the cells are grown under conditions in which the gene product is expressed at a level lower than that of a wild type cell. For example, the cells may be grown under conditions in which a repressor reduces the level of transcription from the regulatable promoter.

In other embodiments, underexpression may be achieved by operably linking the gene required for proliferation to a desired promoter in a vector as described above with respect to embodiments in which gene products required for proliferation are overexpressed. In some embodiments, the vector may be present in cells in which the chromosomal copy or copies of the gene has been disrupted.

Examples of gene products, which are encoded by genes that can be underexpressed using methods such as those described above with respect to methods in which the gene product is overexpressed include a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

One embodiment of the invention includes a method for identifying a gene product described herein on which a compound which inhibits the proliferation of an organism acts. The method employs a culture which comprises a mixture of strains of the organism. At least some of the strains in the culture overexpress a different gene product which is required for the proliferation of the organism. Preferably, each of the strains in the culture overexpresses a different gene product which is required for proliferation of the organism (i. e. all of the strains in the culture overexpress a gene product which is required for proliferation of the organism). For example, the gene product which is overexpressed in each strain may be a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of
SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

Strains that overexpress the proliferation-required gene product may be obtained using the methods described above. The culture may comprise any number of strains which overexpress a gene product required for proliferation. For example the culture may comprise at least two strains, at least 10 strains, at least 20 strains, at least 30, strains, at least 50 strains, at least 100 strains, at least 300 strains or more than 300 strains which overexpress a gene product required for proliferation. In some embodiments, the culture may comprise strains which in aggregate overexpress all or most of the gene products required for proliferation of the organism.

The culture is contacted with a compound which inhibits proliferation of the organism. The compound may be a candidate drug compound obtained from any source. For example, the compound may be a compound generated using combinatorial chemistry, a compound from a natural product library, or an impure or partially purified compound, such as a compound in a partially purified natural extract. The culture is contacted with a sufficient concentration of the compound to inhibit the proliferation of strains of the organism in the culture which do not overexpress the gene product on which the compound acts, such that strains which overexpress said gene product on which the compound acts proliferate more rapidly in the culture than strains which do not overexpress said gene product on which said compound acts. Thus, after a sufficient period of time, the strain which overexpresses the gene product on which the compound acts will be more prevalent in the culture than strains which do not overexpress the gene product on which the compound acts. In a preferred embodiment, the growth conditions and incubation period are selected so that only one strain, the strain overexpressing the target of the compound, is recovered from the culture. Thus, in one embodiment, a plurality of cultures containing a plurality of strains each of which overexpresses a different proliferation-required gene product may be grown in the presence of varying concentrations of the compound. In addition to varying the compound concentrations, in embodiments where expression of the proliferation-required gene product is under the control of a regulatable promoter, the plurality of cultures may be grown at varying concentrations of an agent which regulates the level of expression from the promoter, such as an inducer or an agent which reduces the effect of a repressor on transcription from the promoter. It will be appreciated, that the cultures may be grown in liquid medium in the presence of the compound whose target is to be identified (and where appropriate in the presence of an agent which regulates the level of expression from the promoter) or alternatively, a liquid culture comprising the strains which overexpress the proliferation-required gene products may be grown in the absence of the

compound whose target is to be identified and then introduced onto a solid medium containing the compound (and, where appropriate, also containing an agent which regulates the level of expression from the promoter).

The identity of the overexpressed gene product which is the target of the compound may be determined using a variety of methods. For example, in some embodiments of the present invention, the nucleic acids present in the culture or collection of strains which was contacted with the compound may be compared to the nucleic acids present in a control culture or collection of strains which was not contacted with the compound to identify nucleic acids which are overrepresented in the culture or collection of strains contacted with the test compound relative to the control culture or collection of strains. Alternatively, in some embodiments, the nucleic acids present in a culture or collection of strains contacted with the test compound may be analyzed to identify those nucleic acids which are present without comparison to a control culture or collection of strains.

In some embodiments, the strains which proliferated more rapidly in the culture or collection of strains, i. e. strains having an enhanced ability to proliferate in the presence of a test compound relative to other strains in the culture or collection of strains, are identified as follows.

Amplification products which are correlated with each of the overexpressed genes and which are distinguishable from one another are obtained from a culture or collection grown in the presence of a test compound. The amplification products are distinguished from one another to determine whether a particular amplification product is overrepresented in the culture or collection of strains.

In some embodiments, the amplification products corresponding to each of the gene products have lengths which permit them to be distinguished from one another. In another embodiment, one or more of the amplification products have similar or identical lengths but are distinguishable from one another based on a detectable agent, such as a dye, attached thereto. In some embodiments, amplification products which are overrepresented are identified by comparing the amplification products from the culture or collection of strains which was contacted with the test compound to the amplification products from a culture or collection of strains which was not contacted with the test compound. Alternatively, amplification products which are overrepresented may be identified by simply identifying the amplification products obtained from the culture or collection of strains contacted with the test compound (for example, only one or a few strains may have proliferated in the presence of the test compound). The above methods for generating distinguishable amplification products may be used in conjunction with any of the methods for generating strains which overexpress gene products required for proliferation described herein in order to facilitate the identification of strains which proliferate more rapidly or more slowly in the presence of a test compound.

For example, in some embodiments of the present invention, each of the native promoters of each of the genes encoding gene product required for proliferation are replaced by a single desired replacement promoter. After growth of the culture or collection of strains containing the strains in which the promoters have been replaced in the presence of a test compound for a desired period of time, an amplification reaction is performed on nucleic acids obtained from the culture as follows.

The nucleic acids from the culture or collection of strains may be divided into at least two aliquots if desired. In a preferred embodiment the nucleic acids from the culture or collection of strains are divided into four aliquots. A single primer complementary to a nucleotide sequence within the replacement promoter, within the proliferation required genes, or within nucleic acid sequences adjacent to the promoter or proliferation required genes is divided into at least two portions, one portion for each aliquot of nucleic acids. Each portion of the primer is labeled with a distinct detectable dye, such as the 6FAMTM, TETTE, VICTM, HEXTM, NEDTM, and PET dyes obtainable from Applied Biosystems (Foster City, CA). For example, the DS-31 or DS-33 dye sets available from Applied Biosystems (Foster City, CA) may be used to label the primers.

Alternatively, the HEXTM, NED, JOE, TMR and TETTM dyes available from Amersham
Biosciences may be used. Thus, if the nucleic acids from the culture are not divided into aliquots, a single primer labeled with a single dye may be used. If the nucleic acids from the culture are divided into aliquots, at least 2, at least 3, at least 4 or more than 4 primers labeled with distinguishable dyes may be used. Each of the portions of labeled primers are added to each of the aliquots of the nucleic acids from the culture or collection of strains such that each aliquot of nucleic acid receives a single labeled primer with a single detectable dye thereon. In some embodiments, the primers are divided into 3 portions, 4 portions or

more than 4 portions, with each portion having a dye which is distinguishable from the dyes on the other portions thereon.

Each of the aliquots of nucleic acids also receives a set of unlabeled primers, with each of the unlabeled primers being complementary to a nucleotide sequence within the promoter, within a nucleotide sequence which is unique to one of the genes encoding gene products required for proliferation which were placed under the control of the replacement promoter, or within nucleotide sequences adjacent to the promoter or proliferation required genes. Each of the aliquots receives primers unique to 1/N proliferation required genes which were placed under the control of the replacement promoter, where N is the number of aliquots (i. e. if the culture or collection of strains consisted of 100 strains in which a gene required for proliferation was placed under the control of the replacement promoter and was divided into four aliquots, then each of the four aliquots of nucleic acids from the culture or collection of strains would receive primers complementary to 25 of the genes). The unlabeled primers are selected so that each will yield an amplification product having a length distinguishable from the length of the amplification product produced with the other unlabeled primers. Preferably, the amplification products are between about 100-about 400 nucleotides in length, but any lengths which may be distinguished from each other may be used. In addition, in some of the embodiments some of the amplification products may have identical or very similar lengths but be distinguishable from one another due to labeling with distinguishable dyes.

A nucleic acid amplification reaction is conducted on each of the nucleic acid aliquots. The amplification products are then separated by length to identify amplification products having increased representation in the culture or collection of strains (i. e. amplification products derived from cells which proliferated more rapidly in the culture or collection of strains). The amplification products are then correlated with the corresponding genes to determine which strains proliferated more rapidly in the culture or collection of strains. If desired, amplification products having increased representation in the culture may be identified by comparing the amplification products obtained from a culture or collection of strains which was contacted with the compound to amplification products obtained from a control culture or collection of strains which was not contacted with the compound. Alternatively, if desired, the amplification products which are obtained from a culture which was contacted with the compound may be directly identified without comparison to a control culture which was not contacted with the compound.

For example, in some embodiments, the amplification products from each of the nucleic acid aliquots are pooled and subjected to capillary electrophoresis. The amplification products are detected by detecting the fluorescent dyes attached thereto and their lengths are determined to identify those amplification products having increased or decreased representation in the culture or collection of strains. Figures 2A and 2B illustrate one embodiment of this method in which the absence of an amplification product from an amplification reaction performed on a culture comprising a plurality of strains underexpressing genes required for proliferation indicates that a test compound acts on the gene corresponding to the missing amplification product. It will be appreciated that the method may also be used to identify an amplification product which is overrepresented in an amplification reaction conducted on a culture or collection of strains overexpressing genes required for proliferation because the test compound acted on the corresponding gene.

Alternatively, in another embodiment, a first amplification reaction is performed on nucleic acids obtained from a culture or collection of strains which was contacted with the compound using a first primer complementary to a nucleotide sequence present upstream or downstream of all of the overexpressed genes (such as a primer complementary to a nucleotide sequence in a replacement promoter upstream of all of the overexpressed genes) and a set of primers complementary to a nucleotide sequence unique to each of the strains (such as a primer complementary to a nucleotide sequence within each of the proliferation-required genes). One of the two amplification primers for each of the proliferation required genes is labeled with a dye as described above. Preferably, the common primer complementary to a nucleotide sequence upstream or downstream of all of the overexpressed genes is labeled with the dye. The primers used in the amplification reaction are designed so that the amplification product corresponding to each proliferation-required gene has a unique length or a dye which allows it to be distinguished from other amplification products of the same length. A second amplification reaction is conducted on a control culture or collection of strains which was not contacted with the compound using the same primers as in the first amplification reaction. The amplification products from the first amplification reaction are compared to those from the second amplification reaction to identify one or more amplification products which are overrepresented in the culture or collection of strains. For example, the amplification products from the first amplification reaction may be run in a separate lane of a polyacrylamide gel or a separate capillary than the amplification products from the second amplification reaction and the two lanes or capillaries are compared to one another. If desired, in the embodiment where the amplification products from the first amplification

reaction are run in a different lane or capillary than the amplification products from the second amplification reaction, the same dye may be used to label the primers in the first and second amplification reactions.

Alternatively, if desired, different dyes may be used to label the primers in the first and second amplification reactions. If desired, in the embodiment where the amplification products from the first amplification reaction are run in a different lane or capillary than the amplification products from the second amplification reaction, the same dye may be used to label the primers in the first and second amplification reactions. Alternatively, if desired, different dyes may be used to label the primers in the first and second amplification reactions.

Alternatively, in some embodiments, the primers in the second amplification reaction are labeled with a different dye which is distinguishable from the dye used in the first amplification reaction. In this embodiment, the amplification reactions may be pooled and run in the same lane on a polyacrylamide gel or in the same capillary and the products from each amplification reaction are compared by comparing the amount of each dye present for each amplification product. Figures 3A and 3B illustrate one embodiment of this method in which the absence of an amplification product from the amplification reaction performed on a culture comprising a plurality of strains underexpressing genes required for proliferation which was contacted with the compound indicates that a test compound acts on the gene corresponding to the missing amplification product. It will be appreciated that the method may also be used to identify an amplification product which is overrepresented in an amplification reaction conducted on a culture or collection of strains overexpressing genes required for proliferation because the test compound acted on the corresponding gene.

If desired, rather than dividing the culture into aliquots, individual amplification reactions may be conducted on nucleic acids obtained from the culture or collection of strains. Each amplification reaction contains primers which will yield an amplification product specific for only one of the proliferation required genes. The resulting amplification products from each of the individual amplification reactions are pooled and amplification products having increased representation in the culture are identified as described above.

In another embodiment, a culture or collection of strains in which gene products required for proliferation are overexpressed from regulatable promoters which replaced the native promoters of the genes encoding these gene products is allowed to grow in the presence of a test compound for a desired number of generations. Preferably, the culture or collection of strains is allowed to grow in the presence of the test compound for at least 20 generations. Nucleic acids are isolated from the culture or collection of strains and an amplification reaction is performed using a primer which is complementary to a nucleotide sequence within the replacement promoter (s) or a nucleotide sequence adjacent to the a 5'end thereof and primers which are complementary to a nucleotide sequence within the proliferation required genes or nucleotide sequences adjacent thereto. The resulting amplification product (s) is directly sequenced using a primer complementary to a nucleotide sequence within the replacement promoter.

In one embodiment of the present invention, the vector containing the nucleotide sequence encoding the proliferation-required gene product is obtained from a strain which proliferated more rapidly in the culture using methods such as plasmid preparation techniques. Nucleic acid sequencing techniques are then employed to determine the nucleotide sequence of the gene which was overexpressed.

Alternatively, the identity of the overexpressed gene product which is the target of the compound may be determined by performing a nucleic acid amplification reaction, such as a polymerase chain reaction (PCR), to identify the nucleotide sequence of the gene which was overexpressed. For example, aliquots of a nucleic acid preparation, such as a purified plasmid, from the strain which is recovered from the culture may each be contacted with pairs of PCR primers which would amplify a different proliferation-required gene to determine which pair of primers yields an amplification product.

An alternative method for determining the identity of the gene product described herein which is the target of the compound involves obtaining a nucleic acid array, such as a DNA chip, which contains each of the proliferation-required genes which were overexpressed in the strains in the culture. Each proliferation-required gene occupies a known location in the array. A nucleic acid preparation, such as a plasmid preparation, from the recovered strain is labeled with a detectable agent, such as radioactive or fluorescent moiety, and placed in contact with the nucleic acid array under conditions which permit the labeled nucleic acid to hybridize to complementary nucleic acids on the array. The location on the array to which the labeled nucleic acids hybridize is determined to

identify the gene which was overexpressed in the recovered strain. If desired the hybridized nucleic acids from a culture which was contacted with the compound may be compared to the hybridized nucleic acids from a control culture which was not contacted with the compound.

Alternatively, the hybridized nucleic acids from a culture which was contacted with the compound may be directly identified without comparison to nucleic acids from a control culture.

In some instances, more than one strain may proliferate more rapidly in the presence of the compound. This may result from a variety of causes. For example, the concentration of the compound may not have been high enough to restrict proliferation only to cells which overexpress one gene product (i. e. the target gene product). While strains which overexpress the target gene product will be the most prevalent strain in the culture, other strains may also have proliferated. In such instances, the identity of the gene product in the strain which is most prevalent in the culture may be identified by quantitating the levels of each of the genes encoding proliferation-required proteins in the culture. This may be accomplished by quantitative PCR, DNA sequencing, hybridization, or array technology as described above.

In other instances, multiple strains will exhibit more rapid proliferation in the culture as a result of a common functional attribute. For example, the strains which proliferate more rapidly may each overexpress a gene product with a common enzymatic activity, such as serine protease activity for example. Alternatively, the strains which proliferate more rapidly may each overexpress a gene product with a common functional domain, such as a cAMP binding domain. In such instances, the common attribute of the strains which proliferate more rapidly may provide information as to the mode of action of the compound or the biochemical activity of the target of the compound. For example, if all of the overexpressed genes in the strains which proliferated more rapidly are serine proteases, the compound acts by inhibiting serine protease activity and the target protein is a serine protease. If desired, the compound may be derivatized and the efficacy of the derivatized compound against each of the strains which proliferated more rapidly may be assessed as described herein in order to identify derivatives which are capable of interacting with a wide range of targets sharing a common activity or binding site (i. e. derivatives which have a greater ability to inhibit the proliferation of all the strains than the original compound) or to identify derivatives having greater specificity for a desired target (i. e. derivatives which have a greater specificity for one of the strains than the original compound). For example, it is possible that a nonessential gene product expressed in the cell might also bind to the initial test compound in addition to the gene product required for proliferation. In such an instance, it is desirable to obtain a derivative of the initial test compound which is specific for the gene product required for proliferation. In addition, it is possible that two gene products required for proliferation might bind to the initial test compound but specificity for one of the gene products is desired.

Rather than employing a single culture which contains multiple strains each of which overexpresses a proliferation-required gene product described herein, the methods of the present invention may be performed using an array of individual strains (i. e. a collection of strains) each of which overexpresses a different proliferation-required gene product. For example, individual strains each overexpressing a different proliferation-required gene product may be grown in different wells of a multiwell plate. Each well is contacted with the compound (and, where appropriate an agent which regulates the level of expression from the promoter). The level of proliferation of the strains in each of the wells is determined to identify a strain which proliferated more rapidly. The identity of the overexpressed gene product in the strain that proliferated more rapidly is determined as described above.

In another embodiment, individual strains each overexpressing a different proliferationrequired gene product (i. e. a collection of strains) are grown at different locations on a solid medium, such as an agar plate. The medium contains the compound and where appropriate an agent which regulates the level of expression from the promoter). The level of proliferation of each of the strains is determined to identify a strain which proliferated more rapidly. The identity of the overexpressed gene product in the strain that proliferated more rapidly is determined as described above.

The above methods may be used to prioritize compound development or to determine whether the compound has been previously identified or whether the target of the compound is the target of a previously identified drug. In particular, if the product is a natural product, it is advantageous to determine whether it has been previously identified prior to investing significant effort in developing it. Thus, in some embodiments of the present invention, the target of a partially purified or purified natural product or a compound produced by combinatorial chemistry is identified using the methods described above and compared to the targets of known drugs. If the target is identical to that of a known drug, further development of the compound is halted.

Alternatively, an array of strains each of which overexpresses a different gene product described herein (i. e. a collection of strains) is grown on solid medium containing a compound to be evaluated. The location of each strain in the array and the gene product overexpressed by that strain is grown. The pattern of colonies which grow in the presence of the compound is evaluated and compared to the pattern of colonies which grow in the presence of previously identified drugs. If the pattern of colonies which grow in the presence of the compound being evaluated is the same as the pattern of colonies which grow in the presence of a previously identified drug, further development of the compound is halted.

Additionally in some embodiments, the sequence of the gene product in a strain which proliferated more rapidly in the assays described above is compared to the sequence of gene products from heterologous organisms to determine the likely spectrum of species whose growth would be inhibited by the compound. If the gene product has a high degree of homology to gene products from heterologous species, it is lilcely that the compound would also inhibit the growth of these heterologous species. Homology may be determined using any of a variety of methods familiar to those skilled in the art. For example, homology may be determined using a computer program such as BLASTP or FASTA. The ability of the compound to inhibit the growth of the heterologous species may then be confirmed by comparing the growth of cells of the heterologous species in the presence and absence of the compound.

Current methods for identifying the target of compounds which inhibit cellular proliferation are laborious and time consuming. The above methods may be employed to allow the targets of a large number of compounds to be rapidly identified. In such methods, the methods described above are simultaneously performed for each of a large number of compounds. For example, the compounds may be members of a library of compounds generated using combinatorial chemistry or members of a natural product library. In such methods, a plurality of cultures each comprising a plurality of strains each of which overexpresses a different gene product required for proliferation or a plurality of collections of individual strains each of which overexpresses a different gene product required for proliferation is obtained. Each culture or collection of strains is contacted with a different compound in the library and the target of the compound is identified as described above.

In another embodiment, the gene product described herein on which a compound which inhibits the proliferation of an organism acts is identified using a culture which comprises a mixture of strains of the organism including strains which underexpress a different gene product which is required for proliferation of the organism (i. e. at least some of the strains in the culture underexpress a gene product which is required for proliferation of the organism). Preferably, each of the strains in the culture underexpress a different a gene product which is required for the proliferation of the organism (i. e. all of the strains in the culture underexpress a gene product which is required for the proliferation of the organism). In some embodiments, the culture comprises the least one strain which underexpresses a gene product selected from the group consisting of a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 621442397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

Strains underexpressing the proliferation-required gene products described herein may be obtained using the methods described above. The culture may comprise any number of strains. For example the culture may comprise at least two strains, at least 10 strains, at least 20 strains, at least 30, strains, at least 50 strains, at least 100 strains, at least 300 strains or more than 300 strains which underexpress a gene product required for proliferation. In some embodiments, the strains in the culture in aggregate may underexpress all or most of the gene products required for proliferation of the organism.

The culture is contacted with a compound which inhibits proliferation of the organism. The compound may be a candidate drug compound obtained from any source. For example, the compound may be a compound generated using combinatorial chemistry, a compound from a natural product library, or an impure or partially purified compound, such as a compound in a partially purified natural extract. The culture is contacted with a sufficient concentration of the compound to inhibit the proliferation of strains of the organism in the culture which underexpress the gene product on which the compound acts, such that strains which do not underexpress the gene product on which the compound acts proliferate more rapidly in the culture than strains which do underexpress said gene product on which said compound acts. Thus, after a sufficient period of time, the strain which underexpresses the gene product on which the

compound acts will be less prevalent in the culture than strains which do not underexpress the gene product on which the compound acts. In one embodiment, the growth conditions and incubation period are selected so that only one strain, the strain underexpressing the target of the compound, proliferates at a reduced rate in the culture. In another embodiment, the growth conditions may be selected so that the strain underexpressing the target of the compound is not recovered from the culture. Thus, in one embodiment, a plurality of cultures containing a plurality of strains each of which underexpresses a different proliferation-required gene product may be grown in the presence of varying concentrations of the compound. In addition to varying the compound concentrations, in embodiments where expression of the proliferation-required gene product is under the control of a regulatable promoter, the plurality of cultures may be grown at varying concentrations of an agent which regulates the level of expression from the promoter, such as an inducer or an agent which reduces the effect of a repressor on transcription from the promoter. It will be appreciated, that the cultures may be grown in liquid medium in the presence of the compound whose target is to be identified (and where appropriate in the presence of an agent which regulates the level of expression from the promoter) or alternatively, a liquid culture comprising the strains which underexpress the proliferation-required gene products may be grown in the absence of the compound whose target is to be identified and then introduced onto a solid medium containing the compound (and, where appropriate, also containing an agent which regulates the level of expression from the promoter).

* The identity of the underexpressed gene product which is the target of the compound may be determined using a variety of methods. For example, in some embodiments of the present invention, the nucleic acids present in the culture or collection of strains which was contacted with the compound may be compared to the nucleic acids present in a control culture or collection of strains which was not contacted with the compound to identify nucleic acids which are underrepresented in the culture or collection of strains contacted with the test compound relative to the control culture or strains. Alternatively, in some embodiments, the nucleic acids present in a culture or collection of strains contacted with the test compound may be analyzed to identify those nucleic acids which are missing or present at reduced levels without comparison to a control culture or collection of strains.

In some embodiments of the present invention, the strains which proliferated more slowly in the culture or collection of strains, i. e. strains having an decreased ability to proliferate in the presence of a test compound or which do not proliferate in the presence of a test compound, are identified as follows. Amplification products which are correlated with each of the underexpressed genes and which are distinguishable from one another are obtained from a culture or collection grown in the presence of a test compound. The amplification products are distinguished from one another to determine whether a particular amplification product is. underrepresented in the culture or collection of strains. In some embodiments, the amplification products corresponding to each of the gene products have lengths which permit them to be distinguished from one another. In another embodiment, one or more of the amplification products have similar or identical lengths but are distinguishable from one another based on a detectable agent, such as a dye, attached thereto. In some embodiments, amplification products which are underrepresented are identified by comparing the amplification products from the culture or collection of strains which was contacted with the test compound to the amplification products from a culture or collection of strains which was not contacted with the test compound. Alternatively, amplification products which are underrepresented in the culture or collection of strains may be identified simply by determining which amplification products are missing or present at reduced levels in the culture or collection of strains. The above methods for generating distinguishable amplification products may be used in conjunction with any of the methods for generating strains which underexpress gene products required for proliferation described herein in order to facilitate the identification of strains which proliferate more slowly in the presence of a test compound.

For example, in some embodiments of the present invention, each of the native promoters of each of the genes encoding gene product required for proliferation are replaced by a single desired replacement promoter. After growth of the culture or collection of strains containing the strains in which the promoters have been replaced in the presence of a test compound for a desired period of time, an amplification reaction is performed on nucleic acids obtained from the culture as follows.

The nucleic acids from the culture or collection of strains are divided into at least two aliquots. In a preferred embodiment the nucleic acids from the culture or collection of strains are divided into four aliquots. A single primer complementary to a nucleotide sequence within the replacement promoter, within the proliferation required genes, or within nucleic acid sequences adjacent to the promoter or proliferation required genes is divided into four groups Each group is labeled with a distinct detectable dye, such as the 6FAMTM, TETTE, VICTM, HEXTM, NEDTM, and
PETTM dyes obtainable from Applied Biosystems (Foster City, CA). For example, the DS-31 or

DS-33 dye sets available from Applied Biosystems (Foster City, CA) may be used to label the primers. Each of the groups of labeled primers are added to each of the aliquots of the nucleic acids from the culture or collection of strains such that each aliquot of nucleic acid receives a single labeled primer with a single detectable dye thereon.

Each of the aliquots of nucleic acids also receives a set of unlabeled primers, with each of the unlabeled primers being complementary to a nucleotide sequence within the promoter, within a nucleotide sequence which is unique to one of the genes encoding gene products required for proliferation which were placed under the control of the replacement promoter, or within nucleotide sequences adjacent to the promoter or proliferation required genes. Each of the aliquots receives primers unique to 1/N proliferation required genes which were placed under the control of the replacement promoter, where N is the number of aliquots (i. e. if the culture or collection of strains consisted of 100 strains in which a gene required for proliferation was placed under the control of the replacement promoter and was divided into four aliquots, then each of the four aliquots of nucleic acids from the culture or collection of strains would receive primers complementary to 25 of the genes). The unlabeled primers are selected so that each will yield an amplification product having a length distinguishable from the length of the amplification product produced with the other unlabeled primers. Preferably, the amplification products are between about 100-about 400 nucleotides in length, but any lengths which may be distinguished from each other may be used. In addition, in some of the embodiments some of the amplification products may have identical or very similar lengths but be distinguishable from one another due to labeling with distinguishable dyes.

A nucleic acid amplification reaction is conducted on each of the nucleic acid aliquots. The amplification products are then separated by length to identify amplification products decreased representation or which are absent in the culture or collection of strains. The amplification products are then correlated with the corresponding genes to determine which strains proliferated more slowly in the culture or collection of strains. If desired, amplification products having decreased representation in the culture may be identified by comparing the amplification products obtained from a culture or collection of strains which was contacted with the compound to amplification products obtained from a control culture or collection of strains which was not contacted with the compound. Alternatively, if desired, the amplification products which are missing or present at reduced levels in a culture which was contacted with the compound may be directly identified without comparison to a control culture which was not contacted with the compound.

For example, in some embodiments, the amplification products from each of the nucleic acid aliquots are pooled and subjected to capillary electrophoresis. The amplification products are detected by detecting the fluorescent dyes attached thereto and their lengths are determined to identify those amplification products having decreased representation in the culture or collection of strains. Figures 2A and 2B illustrate one embodiment of this method in which the absence of an amplification product from an amplification reaction performed on a culture comprising a plurality of strains underexpressing genes required for proliferation indicates that a test compound acts on the gene corresponding to the missing amplification product.

Alternatively, in another embodiment, a first amplification reaction is performed on nucleic acids obtained from a culture or collection of strains which was contacted with the compound using a first primer complementary to a nucleotide sequence present upstream or downstream of all of the overexpressed genes (such as a primer complementary to a nucleotide sequence in a replacement promoter upstream of all of the overexpressed genes) and a set of primers complementary to a nucleotide sequence unique to each of the strains (such as a primer complementary to a nucleotide sequence within each of the proliferation-required genes). One of the two amplification primers for each of the proliferation required genes is labeled with a dye as described above. Preferably, the common primer complementary to a nucleotide sequence upstream or downstream of all of the overexpressed genes is labeled with the dye. The primers used in the amplification reaction are designed so that the amplification product corresponding to each proliferation-required gene has a unique length. A second amplification reaction is conducted on a control culture or collection of strains which was not contacted with the compound using the same primers as in the first amplification reaction. The amplification products from the first amplification reaction are compared to those from the second amplification reaction to identify one or more amplification products which are underrepresented in the culture or collection of strains. For example, the amplification products from the first amplification reaction may be run in a separate lane of a polyacrylamide gel or a separate capillary than the amplification products from the second amplification reaction and the two lanes or capillaries are compared to one another.

Alternatively, in some embodiments, the primers in the second amplification reaction are labeled with a different dye which is distinguishable from the dye

used in the first amplification reaction. In this embodiment, the amplification reactions may be pooled and run in the same lane on a polyacrylamide gel or in the same capillary and the products from each amplification reaction are compared by comparing the amount of each dye present for each amplification product. Figures
3A and 3B illustrate one embodiment of this method in which the absence of an amplification product from the amplification reaction performed on a culture comprising a plurality of strains underexpressing genes required for proliferation which was contacted with the compound indicates that a test compound acts on the gene corresponding to the missing amplification product.

If desired, rather than dividing the culture into aliquots, individual amplification reactions may be conducted on nucleic acids obtained from the culture or collection of strains. Each amplification reaction contains primers which will yield an amplification product specific for only one of the proliferation required genes. The resulting amplification products from each of the individual amplification reactions are pooled and amplification products having decreased representation in the culture are identified as described above.

In an alternative embodiment, the representation of each strain in the culture may be assessed by hybridizing detectably labeled nucleic acids encoding the proliferation-required gene products, or portions thereof, obtained from the culture to an array comprising nucleic acids encoding the gene products required for proliferation or portions thereof. Each nucleic acid encoding a gene product required for proliferation or portion thereof occupies a known location on the array. The signal from each location on the array is quantitated to identify those nucleic acids encoding a proliferation-required gene product which are underrepresented in the culture. If desired the hybridized nucleic acids from a culture which was contacted with the compound may be compared to the hybridized nucleic acids from a control culture which was not contacted with the compound. Alternatively, the hybridized nucleic acids from a culture which was contacted with the compound may be directly analyzed without comparison to nucleic acids from a control culture.

In another alternative, each strain underexpressing a gene product required for proliferation may be constructed to contain a unique nucleic acid sequence (referred to herein as a"tag"). The tag may be included in the chromosome of each strain or in an extrachromosomal vector. For example, the tag could be included in a vector encoding an antisense nucleic acid complementary to a gene encoding a gene product required for proliferation or a portion of such a gene or the tag may be included in the antisense nucleic acid itself. The representation of each strain in the culture may be assessed by performing an amplification reaction using primers complementary to each of the tags and quantitating the levels of the resulting amplification products to identify a tag which is underrepresented or absent from the culture. Since each tag corresponds to one strain, the strain which is underrepresented or absent from the culture may be identified. If desired the tags present in a culture which was contacted with the compound may be compared to the tags present in a control culture which was not contacted with the compound. Alternatively, the tags present in a culture which was contacted with the compound may be analyzed without comparison to a control culture.

It will be appreciated that, if desired, unique tags may also be used in embodiments in which gene products required for proliferation are overexpressed. In some aspects of such embodiments, the tags may be within or adjacent to the promoter which drives expression of the gene encoding the gene product. In such embodiments, the gene product which is overexpressed in strains which proliferate more rapidly in the culture may be identified by detecting the presence or amount of the unique tag corresponding to that gene product in the culture.

In some instances, more than one strain may proliferate less rapidly in the presence of the compound. This may result from a variety of causes. For example, the concentration of the compound may not have been high enough to reduce the proliferation only in cells which underexpress one gene product (i. e. the target gene product). While strains which underexpress the target gene product will be the least prevalent strain in the culture, other strains may also be underrepresented. In such instances, the identity of the gene product in the strain which is least prevalent in the culture (or not recovered from the culture) may be identified by quantitating the levels of each of the genes encoding proliferation-required proteins in the culture. This may be accomplished by quantitative PCR, DNA sequencing, hybridization, or array technology as described above.

In other instances, multiple strains will exhibit less rapid proliferation in the culture as a result of a common functional attribute. For example, the strains which proliferate less rapidly (or the strains which are not recovered from the culture) may each underexpress a gene product with a common enzymatic

activity, such as serine protease activity for example. Alternatively, the strains which proliferate less rapidly (or the strains which are not recovered from the culture) may each underexpress a gene product with a common functional domain, such as a cAMP binding domain.

In such instances, the common attribute of the strains which proliferate less rapidly (or the strains which are not recovered from the culture) may provide information as to the mode of action of the compound or the biochemical activity of the target of the compound. For example, if all of the underexpressed genes in the strains which proliferated less rapidly are serine proteases, the compound acts by inhibiting serine protease activity and the target protein is a serine protease. If desired, the compound may be derivatized and the efficacy of the derivatized compound against each of the strains which proliferated more rapidly may be assessed as described herein in order to identify derivatives which are capable of interacting with a wide range of targets sharing a common activity or binding site (i. e. derivatives which have a greater ability to inhibit the proliferation of all the strains than the original compound) or to identify derivatives having greater specificity for a desired target (i. e. derivatives which have a greater specificity for one of the strains than the original compound).

Rather than employing a single culture which contains multiple strains each of which underexpresses a proliferation-required gene product described herein, the methods of the present invention may be performed using an array of individual strains (i. e. a collection of strains) each of which underexpresses a different proliferation-required gene product. For example, individual strains each underexpressing a different proliferation-required gene product may be grown in different wells of a multiwell plate. Each well is contacted with the compound (and, where appropriate an agent which regulates the level of expression from the promoter). The level of proliferation of the strains in each of the wells is determined to identify a strain which proliferated less rapidly or which did not proliferate at all. The identity of the underexpressed gene product in the strain that proliferated less rapidly or which did not proliferate at all is determined as described above.

In another embodiment, individual strains each underexpressing a different proliferationrequired gene product (i. e. a collection of strains) are grown at different locations on a solid medium, such as an agar plate. The medium contains the compound and, where appropriate, an agent which regulates the level of expression from the promoter. The level of proliferation of each of the strains is determined to identify a strain which proliferated less rapidly (or a strain which is not recovered from the culture). The identity of the underexpressed gene product in the strain that proliferated less rapidly (or the strain which is not recovered from the culture) is determined as described above.

The above methods may be used to prioritize compound development or to determine whether the compound has been previously identified or whether the target of the compound is the target of a previously identified drug. In particular, if the product is a natural product is advantageous to determine whether it has been previously identified prior to investing significant effort in developing it. Thus, in some embodiments of the present invention, the target of a partially purified or purified natural product or a compound produced by combinatorial chemistry is identified using the methods described above and compared to the targets of known drugs. If the target is identical to that of a grown drug, further development of the compound is halted.

Alternatively, an array of strains each of which underexpresses a different gene product described herein (i. e. a collection of strains) is grown on solid medium containing a compound to be evaluated. The location of each strain in the array and the gene product underexpressed by that strain is known. The pattern of colonies which grow less rapidly or fail to grow in the presence of the compound is evaluated and compared to the pattern of colonies which grow less rapidly or fail to grow in the presence of previously identified drugs. If the pattern of colonies which grow less rapidly or fail to grow in the presence of the compound being evaluated is the same as the pattern of colonies which grow less rapidly or fail to grow in the presence of a previously identified drug, further development of the compound is halted.

Additionally, the nucleotide sequence of the gene product described herein in a strain which proliferated less rapidly (or a strain which was not recovered from the culture) in the assays described above is compared to the nucleotide sequence of gene products from heterologous organisms to determine the likely spectrum of species whose growth would be inhibited by the compound. If the gene product has a high degree of homology to gene products from heterologous species, it is likely that the compound would also inhibit the growth of these heterologous species.

Homology may be determined using any of a variety of methods familiar to those skilled in the art.

For example, homology may be determined using a computer program such as BLASTP or FASTA. The ability of the compound to inhibit the growth of the heterologous species may then be confirmed by comparing the growth of cells of the heterologous species in the presence and absence of the compound.

In other embodiments, the present invention uses collections or cultures of strains comprising both strains which overexpress gene products described herein required for cellular proliferation and strains which underexpress the same gene products required for cellular proliferation. The gene product which is overexpressed or underexpressed in each strain may be a gene product whose activity or level is inhibited by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a gene product encoded by a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 621442397, a gene product comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a gene product whose activity or level is inhibited by a homologous antisense nucleic acid, a gene product encoded by a homologous coding nucleic acid, and a gene product comprising a homologous polypeptide.

The culture or collection of strains is contacted with a compound and the nucleic acids present in the culture or collection of strains are analyzed. Preferably, nucleic acids derived from overexpressing strains can be distinguished from those derived from underexpressing strains. For example, the overexpressing strains may be obtained using promoter replacement as described above while the underexpressing strains may be obtained by expressing antisense nucleic acids.

Accordingly, in one embodiment, amplification primers may be designed which will uniquely amplify nucleic acids from the overexpressing strains or the underexpressing strains. If a compound acts on a gene product which was overexpressed and underexpressed in the culture, then the amplification product obtained from the strain in the culture or collection which overexpressed gene product will be overrepresented in the culture or collection while the amplification product obtained from the strain which underexpressed the gene product will be underrepresented in the culture or collection. If desired, nucleic acids from a culture or collection which was contacted with the compound may be compared to nucleic acids from a control culture or collection which was not contacted with the compound. Alternatively, nucleic acids from a culture or collection which was contacted with the compound may be directly analyzed without comparison to a control culture or collection.

In some embodiments, strains are constructed in which a nucleic acid complementary to a gene encoding a gene product described herein required for proliferation or a portion thereof is operably linked to a regulatable promoter. For example, in some embodiments, the strains may transcribe an antisense nucleic acid selected from the group consisting of SEQ ID NOs.: 1-6213 or fragments thereof which inhibit proliferation or reduce the activity or level of the gene product encoded by the gene comprising a nucleotide sequence complementary to the antisense nucleic acid or homologous antisense nucleic acids or fragments thereof. In other embodiments, the strains may transcribe an antisense nucleic acid which reduces the activity or level of a gene product encoded by SEQ ID NOs.: 6214-42397, the polypeptides of SEQ ID NOs.: 42398-78581, homologous coding nucleic acids or homologous polypeptides. A culture comprising a plurality of such strains wherein each strain expresses an antisense nucleic acid against a different gene product required for proliferation is grown in the presence of varying levels of a compound which inhibits proliferation and in the presence of varying levels of an agent which regulates the level of transcription from the regulatable promoter. Nucleic acids samples are obtained from the culture, detectably labeled and hybridized to a solid support comprising nucleic acids containing the genes encoding the proliferation-required gene products or a portion thereof. The level of hybridization is quantitated for each nucleic acid encoding each of the proliferation-required gene products to determine the rate at which each of the strains proliferated in the culture. If the antisense nucleic acid expressed by a strain in the culture is not complementary to all or a portion of the gene encoding the target of the compound (i. e. a nonspecific strain), then the hybridization intensity for that strain will not be correlated with the concentration of the compound (See Figure 4), while if the antisense nucleic acid expressed by a strain in the culture is complementary to all or a portion of the gene encoding the target of the compound, the hybridization intensity for that strain will be intimately correlated with the concentration of the compound (See Figure 5). In this manner, the target of the compound may be identified. It will be appreciated that, as described above, rather than growing the strains in a single culture, each strain may be grown in a different location on a solid medium or in a different well of a multiwell plate.

The methods described above can be simultaneously performed for each of a large number of compounds. For example, the compounds may be members of a library of compounds generated using combinatorial chemistry or members of a natural product library. In such methods, a plurality of cultures each comprising a plurality of strains each of which overexpresses or underexpresses a different gene product required for proliferation or a plurality of collections of individual strains each of which overexpresses or underexpresses a different gene product required for proliferation is obtained. Each culture or collection of strains is contacted with a different compound in the library and the target of the compound is identified as described above.

In still another embodiment, the antisense nucleic acids of the present invention (including the antisense nucelic acids of SEQ ID NOs. 1-6213 fragments thereof or homologous antisense nucleic acids or fragements thereof) that inhibit bacterial growth or proliferation can be used as antisense therapeutics for killing bacteria. The antisense sequences can be complementary to one of SEQ ID
NOs.: 6214-42397 or fragments thereof, homologous coding nucleic acids or fragments thereof.

Alternatively, antisense therapeutics can be complementary to operons in which proliferation-required genes reside (i. e. the antisense nucleic acid may hybridize to a nucleotide sequence of any gene in the operon in which the proliferation-required genes reside). Further, antisense therapeutics can be complementary to a proliferation-required gene or portion thereof with or without adjacent noncoding sequences, an intragenic sequence (i. e. a sequence within a gene), an intergenic sequence (i. e. a sequence between genes), a sequence spanning at least a portion of two or more genes, a 5'noncoding region or a 3'noncoding region located upstream or downstream from the actual sequence that is required for bacterial proliferation or an operon containing a proliferation-required gene.

In addition to therapeutic applications, the present invention encompasses the use of nucleic acids complementary to nucleic acids required for proliferation as diagnostic tools. For example, nucleic acid probes comprising nucleotide sequences complementary to proliferation-required sequences that are specific for particular species of cells or microorganisms can be used as probes to identify particular microorganism species or cells in clinical specimens. This utility provides a rapid and dependable method by which to identify the causative agent or agents of a bacterial infection. This utility would provide clinicians the ability to accurately identify the species responsible for the infection and amdminister a compound effective against it. In an extension of this utility, antibodies generated against proteins translated from mRNA transcribed from proliferation-required sequences can also be used to screen for specific cells or microorganisms that produce such proteins in a speciesspecific manner.

Other embodiments of the present invention include methods of identifying compounds which inhibit the activity of gene products required for cellular proliferation using rational drug design. As discussed in more detail below, in such methods, the structure of the gene product is determined using techniques such as x-ray crystallography or computer modeling. Compounds are screened to identify those which have a structure which would allow them to interact with the gene product or a portion thereof to inhibit its activity. The compounds may be obtained using any of a variety of methods familiar to those skilled in the art, including combinatorial chemistry. In some embodiments, the compounds may be obtained from a natural product library. In some embodiments, compounds having a structure which allows them to interact with the active site of a gene product, such as the active site of an enzyme, or with a portion of the gene product which interacts with another biomolecule to form a complex are identified. If desired, lead compounds may be identified and further optimized to provide compounds which are highly effective against the gene product.

The following examples teach the genes of the present invention and a subset of uses for the genes identified as required for proliferation. These examples are illustrative only and are not intended to limit the scope of the present invention.

EXAMPLES

The following examples are directed to the identification and exploitation of genes required for proliferation. Methods of gene identification are discussed as well as a variety of methods to utilize the identified sequences. It will be appreciated that any of the antisense nucleic acids, proliferartionrequired genes or proliferation-required gene products described herein, or portions thereof, may be used in the procedures described below, including the antisense nucleic acids of SEQ ID NOs.: 1-6213, the nucleic acids of SEQ ID NOS.: 6214-42397, or the polypeptides of SEQ ID NOs.: 42398-78581.

Lilcewise, homologous antisense nucleic acids, homologous coding nucleic acids, homologous polypeptides or portions of any of the above-mentioned nucleic acids or polypeptides, may be used in any of the procedures described below.

Genes Identified as Required for Proliferation of Escheiichia coli, Stapltylococcus aureus, Eitterococcusfaecalis, I (lebsiellapiieunioniae, Pseudonionas aeruginosa and Salillonella typhimurium.

Genomic fragments were operably linked to an inducible promoter in a vector and assayed for growth inhibition activity. Example 1 describes the examination of a library of genomic fragments cloned into vectors comprising inducible promoters. Upon induction with xylose or IPTG, the vectors produced an RNA molecule corresponding to the subcloned genomic fragments. In those instances where the genomic fragments were in an antisense orientation with respect to the promoter, the transcript produced was complementary to at least a portion of an mRNA (messenger RNA) encoding a Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa or Salnaoonella typhiinuriunz gene product such that they interacted with sense mRNA produced from various Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumonae, Pseudomonase aeruginosa or Salmonella typhimurium genes and thereby decreased the translation efficiency or the level of the sense messenger RNA thus decreasing production of the protein encoded by these sense mRNA molecules. In cases where the sense mRNA encoded a protein required for proliferation, bacterial cells containing a vector from which transcription from the promoter had been induced failed to grow or grew at a substantially reduced rate.

Additionally, in cases where the transcript produced was complementary to at least a portion of a non translated RNA and where that non-translated RNA was required for proliferation, bacterial cells containing a vector from which transcription from the promoter had been induced also failed to grow or grew at a substantially reduced rate. In contrast, cells grown under non-inducing conditions grow at a normal rate.

The above method was used to identify genes required for cellular proliferation in Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomo7las aeruginosa and Salraonella typhimurium. Additionally, a number of genes required for cellular proliferation in Eschericlaia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa and Salmonella typhimurium, which have been described in the following U. S. Patent Applications: U. S. Patent Application Serial Number 09/492, 709, filed
January 27,2000; U. S. Patent Application Serial Number 09/711,164, filed November 9,2000;
U. S. Patent Application Serial Number 09/741, 669, filed December 19,2000 and U. S. Patent
Application Serial Number 09/815,242 filed March 21,2001, U. S. Provisional Patent Application
Serial Number 60/342, 923, filed October 25,2001, have been previously identified using the above method.

EXAMPLE 1
Inhibition of Bacterial Proliferation after Induction of Antisense Expression
To identify genes required for proliferation of E. coli, random genomic fragments were cloned into the IPTG-inducible expression vector pLEX5BA (Krause et al., J. Mol. Biol. 274: 365 (1997) or a modified version of pLEX5BA, pLEX5BA-3'in which a synthetic linker containing a
T7 terminator was ligated between the PstI and HindIII sites of pLEX5BA. In particular, to construct pLEX5BA-3', the following oligonucleotides were annealed and inserted into the PstI and
HindIII sites of pLEX5BA :
EMI197.1

Random fragments of E. coli genomic DNA were generated by DNAsel digestion or sonication, filled in with T4 polymerase, and cloned into the SmaI site of pLEX5BA or pLEX5BA- 3'. Upon activation or induction, the promoter transcribed the random genomic fragments.

A number of vectors which allow the production of transcripts which have an extended lifetime in E. coli as well as other Gram negative bacteria can also be utilized in conjunction with these antisense inhibition experiments. Such vectors are described in U. S. Provisional Patent

Application Serial Number 60/343,512, filed December 21,2001. Briefly, the stabilized antisense RNA may comprise an antisense RNA which was identified as inhibiting proliferation as described above which has been engineered to contain at least one stem loop flanking each end of the antisense nucleic acid. In some embodiments, the at least one stem-loop structure formed at the 5' end of the stabilized antisense nucleic acid comprises a flush, double stranded 5'end. In some embodiments, one or more of the stem loops comprises a rho independent terminator. In additional embodiments, the stabilized antisense RNA lacks a ribosome binding site. In further embodiments, the stabilized RNA lacks sites which are cleaved by one or more RNAses, such as RNAse E or RNAse III. In some embodiments, the stabilized antisense RNA may be transcribed in a cell which the activity of at least one enzyme involved in RNA degradation has been reduced. For example, the activity of an enzyme such as RNase E, RNase II, RNase III, polynucleotide phosphorylase, and poly (A) polymerase, RNA helicase, enolase or an enzyme having similar functions may be reduced in the cell.

To study the effects of transcriptional induction in liquid medium, growth curves were carried out by back diluting cultures 1: 200 into fresh media with or without 1 rnM IPTG and measuring the OD4so every 30 minutes (min). To study the effects of transcriptional induction on solid medium, 102, 103, 104, 105, 106, 107 and 108 fold dilutions of overnight cultures were prepared. Aliquots of from 0.5 to 3 ul of these dilutions were spotted on selective agar plates with or without 1 mM IPTG. After overnight incubation, the plates were compared to assess the sensitivity of the clones to IPTG.

Of the numerous clones tested, some clones were identified as containing a sequence that inhibited E. coli growth after IPTG induction. Accordingly, the gene to which the inserted nucleic acid sequence corresponds, or a gene within the operon containing the inserted nucleic acid, is required for proliferation in E. coli.

Nucleic acids involved in proliferation of Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumo7tiae, Pseudomonas aeruginosa and Salmonella typhimurium were identified as follows. Randomly generated fragments of Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa or Salmonella typhimurium genomic DNA were transcribed from inducible promoters.

In the case of Staphylococcus aureus, a novel inducible promoter system, XylT5, comprising a modified T5 promoter fused to the xyl0 operater from the xylA promoter of Staphylococcus aureus was used. The promoter is described in U. S. Patent Application Serial Number 10/032,393, filed December 21,2001. Transcription from this hybrid promoter is inducible by xylose.

Randomly generated fragments of Sahnoizella typhimurium genomic DNA were transcribed from an IPTG inducible promoter in pLEX5BA (brause et al., J. Mol. Biol. 274: 365 (1997) or a derivative thereof. Randomly generated fragements of Klebsiella pneumoniae genomic DNA were expressed from an IPTG inducible promoter in pLEX5BA-Kan. To construct pLEX5BA-kan, pLEX5BA was digested to completion with ClaI in order to remove the bla gene.

Then the plasmid was treated with a partial NotI digestion and blunted with T4 DNA polymerase. A 3.2 kbp fragment was then gel purified and ligated to a blunted 1.3 lcbp kan gene from pflanzt Kan resistant transfonnants were selected on Kan plates. Orientation of the kan gene was checlced by HI digestion. A clone, which had the kan gene in the same orientation as the bla gene, was used to identify genes required for proliferation of Klebsiella pneumoniae. Randomly generated fragments of Pseudo7nonas aeruginosa genomic DNA were trancribed from a two-component inducible promoter system. Integrated on the chromosome was the T7 RNA polymerase gene regulated by lacW51 lacO (Brunschwig, E. and Darzins, A. 1992. Gene 111: 35-41. On a separate plasmid, a T7 gene 10 promoter, which is transcribed by T7 RNA polymerase, was fused with a lac0 operator followed by a multiple cloning site.

Should the genomic DNA downstream of the promoter contain, in an antisense orientation, at least a portion of an mRNA or a non-translated RNA encoding a gene product involved in proliferation, then induction of transcription from the promoter will result in detectable inhibition of proliferation.

In the case of Staplzylococcus aureus, a shotgun library of Staplzylococcus aureus genomic fragments was cloned into the vector pXyIT5-P15a, which

harbors the XyIT5 inducible promoter.

The vector was linearized at a unique BamEtI site immediately downstream of the XyIT5 promoter/operator. The linearized vector was treated with shrimp alkaline phosphatase to prevent reclosure of the linearized ends. Genomic DNA isolated from Staphylococcus aureus strain RN450 was fully digested with the restriction enzyme Sau3A, or, alternatively, partially digested with
DNase I and"blunt-ended"by incubating with T4 DNA polymerase. Random genomic fragments between 200 and 800 base pairs in length were selected by gel purification. The size-selected genomic fragments were added to the linearized and dephosphorylated vector at a molar ratio of 0.1 to 1, and ligated to form a shotgun library.

The ligated products were transformed into electrocompetent E. coli strain XL 1-Blue MRF (Stratagene) and plated on LB medium with supplemented with carbenicillin at 100 llg/ml.

Resulting colonies numbering 5 x 105 or greater were scraped and combined, and were then subjected to plasmid purification.

The purified library was then transformed into electrocompetent Staphylococcus aureus
RN4220. Resulting transformants were plated on agar containing LB + 0.2% glucose (LBG medium) + chloramphenicol at 15, ug/ml (LBG+CM15 medium) in order to generate 100 to 150 platings at 500 colonies per plating. The colonies were subjected to robotic picking and arrayed into wells of 384 well culture dishes. Each well contained 100111 of LBG + CM15 liquid medium.

Inoculated 384 well dishes were incubated 16 hours at 37 C, and each well was robotically gridded onto solid LBG + CM15 medium with or without 2% xylose. Gridded plates were incubated 16 hours at 37 C, and then manually scored for arrayed colonies that were growth-compromised in the presence of xylose.

Arrayed colonies that were growth-sensitive on medium containing 2% xylose, yet were able to grow on similar medium lacking xylose, were subjected to further growth sensitivity analysis as follows: Colonies from the plate lacking xylose were manually picked and inoculated into individual wells of a 96 well culture dish containing LBG + CM15, and were incubated for 16 hours at 37 C. These cultures were robotically diluted 1/100 into fresh medium and allowed to incubate for 4 hours at 37 C, after which they were subjected to serial dilutions in a 384 well array and then gridded onto media containing 2% xylose or media lacking xylose. After growth for 16 hours at 37 C, the arrays that resulted on the two media were compared to each other. Clones that grew similarly at all dilutions on both media were scored as a negative and were no longer considered. Clones that grew on xylose medium but failed to grow at the same serial dilution on the non-xylose plate were given a score based on the differential, i. e. should the clone grow at a serial dilution of 104 or less on the xylose plate and grow at a serial dilution of 10 or less on the non-xylose plate, then the corresponding clone received a score of"4"representing the log difference in growth observed.

For Salmonella typlaiynurium and Klebsiella pneumoniae growth curves were carried out by back diluting cultures 1: 200 into fresh media containing 1 mM IPTG or media lacking IPTG and measuring the OD4so every 30 minutes (min). To study the effects of transcriptional induction on solid medium, 102, 103, 104, 105, 106, 107 and 108 fold dilutions of overnight cultures were prepared.

Aliquots of from 0.5 to 3 ul of these dilutions were spotted on selective agar plates with or without 1 mM IPTG. After overnight incubation, the plates were compared to assess the sensitivity of the clones to IPTG.

Nucleic acids involved in proliferation of Pseudomonas aeruginosa were identified as follows. Randomly generated fragments of Pseudomonas aeruginosa genomic DNA were transcribed from a two-component inducible promoter system. Integrated on the chromosome was the T7 RNA polymerase gene regulated by/acUV5//ae0 (Brunschwig, E. and Darzins, A. 1992.

Gene 111: 35-41). On an expression plasmid there was a T7 gene 10 promoter, which is transcribed by T7 RNA polymerase, fused with a lac0 operator

followed by a multiple cloning site.

Transcription from this hybrid promoter is inducible by IPTG. Should the genomic DNA downstream of the promoter contain, in an antisense orientation, at least a portion of an mRNA encoding a gene product involved in proliferation, then induction of expression from the promoter will result in detectable inhibition of proliferation.

A shotgun library of Pseudomonas aeruginosa genomic fragments was cloned into the vectors pEP5, pEP5S, or other similarly constructed vectors which harbor the T71acO inducible promoter. The vector was linearized at a unique SmaI site immediately downstream of the T71acO promoter/operator. The linearized vector was treated with shrimp alkaline phosphatase to prevent reclosure of the linearized ends. Genomic DNA isolated from Pseudomonas aeruginosa strain PAO1 was partially digested with DNase I and"blunt-ended"by incubating with T4 DNA polymerase. Random genomic fragments between 200 and 800 base pairs in length were selected by gel purification. The size-selected genomic fragments were added to the linearized and dephosphorylated vector at a molar ratio of 2 to 1, and ligated to form a shotgun library.

The ligated products were transformed into electrocompetent E, coli strain XLl-Blue MRF (Stratagene) and plated on LB medium with carbenicillin at 100 g/ml or Streptomycin 100 pLg/ml.

Resulting colonies numbering 5 x 105 or greater were scraped and combined, and were then subjected to plasmid purification.

The purified library was then transformed into electrocompetent Pseudomonas aeruginosa strain PAO1. Resulting transformants were plated on LB agar with carbenicillin at 100, ug/ml or
Streptomycin 40 llg/ml in order to generate 100 to 150 platings at 500 colonies per plating. The colonies were subjected to robotic picking and arrayed into wells of 384 well culture dishes. Each well contained 100 ofLB + CB 100 or Streptomycin 40 liquid medium. Inoculated 384 well dishes were incubated 16 hours at room temperature, and each well was robotically gridded onto solid LB + CB100 or Streptomycin 40 medium with or without 1 mM IPTG. Gridded plates were incubated 16 hours at 37 C, and then manually scored for arrayed colonies that were growthcompromised in the presence of IPTG.

Arrayed colonies that were growth-sensitive on medium containing 1 mM IPTG, yet were able to grow on similar medium lacking IPTG, were subjected to further growth sensitivity analysis as follows: Colonies from the plate lacking IPTG were manually picked and inoculated into individual wells of a 96 well culture dish containing LB + CB100 or Streptomycin 40, and were incubated for 16 hours at 30 C. These cultures were robotically diluted 1/100 into fresh medium and allowed to incubate for 4 hours at 37 C, after which they were subjected to serial dilutions in a 384 well array and then gridded onto media with and without 1 mM IPTG. After growth for 16 hours at 37 C, the arrays of serially diluted spots that resulted were compared between the two media. Clones that grew similarly at all dilutions on both media were scored as a negative and were no longer considered. Clones that grew on IPTG medium but failed to grow at the same serial dilution on the non-IPTG plate were given a score based on the differential, i. e. should the clone grow at a serial dilution of 104 or less on the IPTG plate and grow at a serial dilution of 108 or less on the IPTG plate, then the corresponding clone received a score of"4"representing the log difference in growth observed.

Following the identification of those vectors that, upon induction, negatively impacted
Pseudomonas aeruginosa growth or proliferation, the inserts or nucleic acid fragments contained in those vectors were isolated for subsequent characterization. Vectors of interest were subjected to nucleic acid sequence determination.

Nucleic acids involved in proliferation of E. faecalis were identified as follows. Randomly generated fragments of genomic DNA were expressed from the vectors pEPEF3 or pEPEF14, which contain the CP25 or P59 promoter, respectively, regulated by the xyl operator/repressor.

These plasmids as well as other vectors useful for the expression of nucleic acids in Enterococcus faecalis and other Gram positive organisms are described in U. S. Patent Application Serial Number
10/032,393, filed December 21,2001, the disclosure or which is incorportated herein by reference in its entirety. Should the genomic DNA downstream of the

promoter contain, in an antisense orientation, at least a portion of a mRNA encoding a gene product involved in proliferation, then induction of expression from the promoter will result in detectable inhibition of proliferation.

A shotgun library of E. faecalis genomic fragments was cloned into the vector pEPEF3 or pEPEF14, which harbor xylose inducible promoters. The vector was linearized at a unique SinaI site immediately downstream of the promoter/operator. The linearized vector was treated with alkaline phosphatase to prevent reclosure of the linearized ends. Genomic DNA isolated from E. faecalis strain OG1RF was partially digested with DNase I and"blunt-ended"by incubating with
T4 DNA polymerase. Random genomic fragments between 200 and 800 base pairs in length were selected by gel purification. The size-selected genomic fragments were added to the linearized and dephosphorylated vector at a molar ratio of 2 to 1, and ligated to form a shotgun library.

The ligated products were transformed into electrocompetent E. coli strain TOP10 cells (Invitrogen) and plated on LB medium with erythromycin (Enn) at 150 llg/ml. Resulting colonies numbering 5 x 105 or greater were scraped and combined, and were then subjected to plasmid purification.

The purified library was then transformed into electrocompetent E. faecalis strain OG1RF.

Resulting transformants were plated on Todd-Hewitt (TH) agar with erythromycin at 10 u. g/ml in order to generate 100 to 150 platings at 500 colonies per plating. The colonies were subjected to robotic picking and arrayed into wells of 384 well culture dishes. Each well contained 100 ul of
THB + Erin 10 g/ml. Inoculated 384 well dishes were incubated 16 hours at room temperature, and each well was robotically gridded onto solid TH agar + Erm with or without 5% xylose.

Gridded plates were incubated 16 hours at 37 C, and then manually scored for arrayed colonies that were growth-compromised in the presence of xylose.

Arrayed colonies that were growth-sensitive on medium containing 5% xylose, yet were able to grow on similar medium lacking xylose, were subjected to further growth sensitivity analysis. Colonies from the plate lacking xylose were manually picked and inoculated into individual wells of a 96 well culture dish containing THB + Erm 10, and were incubated for 16 hours at 30 C. These cultures were robotically diluted 1/100 into fresh medium and allowed to incubate for 4 hours at 37 C, after which they were subjected to serial dilution on plates containing
5% xylose or plates lacking xylose. After growth for 16 hours at 37 C, the arrays of serially diluted spots that resulted were compared between the two media. Colonies that grew similarly on both media were scored as a negative and corresponding colonies were no longer considered. Colonies on xylose medium that failed to grow to the same serial dilution compared to those on the nonxylose plate were given a score based on the differential. For example, colonies on xylose medium that only grow to a serial dilution of-4 while they were able to grow to-8 on the non-xylose plate, then the corresponding transformant colony received a score of"4"representing the log difference in growth observed.

Following the identification of those vectors that, upon induction, negatively impacted E. faecalis growth or proliferation, the inserts or nucleic acid fragments contained in those expression vectors were isolated for subsequent characterization. The inserts in the vectors of interest were subjected to nucleotide sequence determination.

It will be appreciated that other restriction enzymes and other endonucleases or methodologies may be used to generate random genomic fragments. In addition, random genomic fragments may be generated by mechanical shearing. Sonication and nebulization are two such techniques commonly used for mechanical shearing of DNA.

EXAMPLE 2
Nucleotide Sequence Determination of Identified Clones Transribing Nucleic Acid Fragments with
Detrimental Effects on Escherichia coli. Staphococcus aureus. Enterococcus faecalis. Klebsiella pneumoniae Pseudomonas aerug nosa or Salmonella typhimurium Proliferation

Plasmids from clones that received a dilution plating score of"2"or greater were isolated to obtain the genomic DNA insert responsible for growth inhibition as follows.

The nucleotide sequences of the nucleic acid sequences which inhibited the growth of
Escherichia coli were determined using plasmid DNA isolated using QIAPREP (Qiagen, Valencia,
CA) and methods supplied by the manufacturer. The primers used for sequencing the inserts were 5'
TGTTTATCAGACCGCTT-3' (SEQ ID NO: 78586) and 5'-ACAATTTCACACAGCCTC-3' (SEQ ID NO: 78587). These sequences flank the polylinlcer in pLEX5BA.

The nucleotide sequences of the nucleic acid sequences which inhibited the growth of
Staphylococcus aureus were determined as follows. Staphylococcus aureus were grown in standard laboratory media (LB or TB with 15 ug/ml Chloramphenicol to select for the plasmid). Growth was carried out at 37 C overnight in culture tubes or 2 ml deep well microtiter plates.

Lysis of Staphylococcus aureus was performed as follows. Cultures (2-5 ml) were centrifuged and the cell pellets resuspended in 1.5 mg/ml solution of lysostaphin (20 Rl/ml of original culture) followed by addition of 250 1ll of resuspension buffer (Qiagen). Alternatively, cell pellets were resuspended directly in 250 l of resuspension buffer (Qiagen) to which 5-20 p1 of a 1 mg/ml lysostaphin solution were added.

DNA was isolated using Qiagen miniprep kits or Wizard (Qiagen) miniprep lcits according to the instructions provided by the manufacturer.

The genomic DNA inserts were amplified from the purified plasmids by PCR as follows.

1 u. of Qiagen purified plasmid was put into a total reaction volume of 25 1ll Qiagen Hot
Start PCR mix. For Staphylococcus aureus, the following primers were used in the PCR reaction: pXylT5F : CAGCAGTCTGAGTTATAAAATAG (SEQ ID NO : 78588)
LexL TGTTTTATCAGACCGCTT (SEQ ID NO: 78589)
Similar methods were conducted for Salmonella typhi7nurium and Klebsiella pneumoniae.

For Salmonella typlaimuriurra and Klebsiella pneumoniae the following primers were used: 5'-TGTTTTATCAGACCGCTT-3' (SEQ ID NO: 78589) and 5'-ACAATTTCACACAGCCTC-3' (SEQ ID NO: 78587)
PCR was carried out in a PE GenAmp with the following cycle times:
Step 1. 95'C 15 min
Step 2.94 C 45 sec Step 3. 54'C 45 sec
Step 4.72 C 1 minute
Step 5. Return to step 2,29 times
Step 6.72 C 10 minutes
Step 7.4 C hold
The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

For Pseudomonas aeruginosa, plasmids from transformant colonies that received a dilution plating score of"2"or greater were isolated to obtain the genomic DNA insert responsible for growth inhibition as follows. Pseudomonas aeruginosa were grown in standard laboratory media (LB with carbenicillin at 100 llg/ml or Streptomycin 40 Lg/i-nl to select for the plasmid). Growth was carried out at 30 C overnight in 100 ul culture wells in microtiter plates. To amplify insert
DNA 2 ul of culture were placed into 25 ul Qiagen Hot Start PCR mix. PCR reactions were in 96 well microtiter plates. For plasmid pEP5S the following primers were used in the PCR reaction: T7L1+ : GTCGGCGATATAGGCGCCAGCAACCG (SEQ ID NO: 78590) pStrA3:

ATAATCGAGCATGAGTATCATACG (SEQ ID NO: 78591)
PCR was carried out in a PE GenAmp with the following cycle times:
Step 1.95'C 15 min Step 2. 94'C 45 sec
Step 3.54 C 45 sec
Step 4.72 C 1 minute
Step 5. Return to step 2,29 times
Step 6.72 C 10 minutes
Step 7.4 C hold
The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

The purified PCR products were then directly cycle sequenced with Qiagen Hot Start PCR mix. The following primers were used in the sequencing reaction:
T7/L2: ATGCGTCCGGCGTAGAGGAT (SEQ ID NO: 78592)
PCR was carried out in a PE GenAmp with the following cycle times:
Step 1.94'C 15 min
Step 2.96 C 10 sec
Step 3.50 C 5 sec
Step 4.60 C 4 min
Step 5. Return to step 2,24 times
Step 6.4 C hold
The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

For E. faecalis, plasmids from transformant colonies that received a dilution plating score of"2"or greater were isolated to obtain the genomic DNA insert responsible for growth inhibition as follows. E. faecalis were grown in THB 10 n. g/ml Erm at 30 C overnight in 100 ul culture wells in microtiter plates. To amplify insert DNA 2 ul of culture were placed into 25 1ll Qiagen Hot Start
PCR mix. PCR reactions were in 96 well microtiter plates. The following primers were used in the
PCR reaction: pXylT5 : CAGCAGTCTGAGTTATAAAATAG (SEQ ID NO: 78588) and the pEP/pAKl primer.

PCR was carried out in a PE GenAmp with the following cycle times:
Step 1.95'C 15 min
Step 2.94 C 45 sec
Step 3.54 C 45 sec
Step 4.72 C 1 minute
Step 5. Return to step 2,29 times
Step 6.72 C 10 minutes
Step 7.4 C hold
The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

The purified PCR products were then directly cycle sequenced with Qiagen Hot Start PCR mix. The following primers were used in the PCR reaction:
pXylT5 : CAGCAGTCTGAGTTATAAAATAG (SEQ ID NO: 78588)
PCR was carried out in a PE GenAmp with the following cycle times:
Step 1.94 C 15 min
Step 2.96 C 10 sec Step 3. 50 C 5 sec
Step 4.60 C 4 min
Step 5. Return to step 2,24 times

Step 6.4 C hold
The PCR products were cleaned using Qiagen Qiaquick PCR plates according to the manufacturer's instructions.

The amplified genomic DNA inserts from each of the above procedures were subjected to automated sequencing. Sequence identification numbers (SEQ ID NOs) and clone names for the identified inserts are listed in Table IA and discussed below.

TABLE IA
EMI206.1

EMI207.1

EMI208.1

EMI209.1

EMI210.1

EMI211.1

EMI212.1

EMI213.1

EMI214.1

EMI215.1

EMI216.1

EMI217.1

EMI218.1

EMI219.1

EMI220.1

EMI221.1

EMI222.1

EMI223.1

EMI224.1

EMI225.1

EMI226.1

EMI227.1

EMI228.1

EMI229.1

EMI230.1

EMI231.1

EMI232.1

EMI233.1

EMI234.1

EMI235.1

EMI236.1

EMI237.1

EMI238.1

EMI239.1

EMI240.1

EXAMPLE 3

Comparison Of Isolated Nucleic Acids to Known Sequences

The nucleotide sequences of the subcloned fragments from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsilella pneumoniae Pseudomonas aeruginosa and Salinonella typhimurium obtained from the expression vectors discussed above were compared to known sequences from Escherichia coli, Staplaylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium and other microorganisms as follows. First, to confirm that each clone originated from one location on the chromosome and was not chimeric, the nucleotide sequences of the selected clones were compared against the

Escherichia coli, Staphylococcus aureus, Eraterococcus faecalis, Klebsiella pyaeumoniae, Pseudo7nonas ae-uginosa or Salnzonella typhisouriunn genomic sequences to align the clone to the correct position on the chromosome. The NCBI BLASTN v 2.0.9 program was used for this comparison, and the incomplete Staphylococus aureus genomic sequences licensed from TIGR, as well as the NCBI nonredundant GenBank database were used as the source of genomic data.

Salmonella typhimurium sequences were compared to sequences available from the Genome

Sequencing Center (http ://genome. wustl. edu/gsc/salmonella. shtml), and the Sanger Centre (http://www. sanger. ac. ul/projects/S typhi). Pseudomonas aemgizosa sequences were compared to a proprietary database and the NCBI GenBank database. The E. faecalis sequences were compared to a proprietary database.

The BLASTN analysis was performed using the default parameters except that the filtering was turned off. No further analysis was performed on inserts which resulted from the ligation of multiple fragments.

In general, antisense molecules and their complementary genes are identified as follows.

First, all possible full length open reading frames (ORFs) are extracted from available genomic databases. Such databases include the GenBank nonredundant (nr) database, the unfinished genome database available from TIGR and the PathoSeq database developed by Incyte Genomics.

The latter database comprises over 40 annotated bacterial genomes including complete ORF analysis. If databases are incomplete with regard to the bacterial genome of interest, it is not necessary to extract all ORFs in the genome but only to extract the ORFs within the portions of the available genomic sequences which are complementary to the clones of interest. Computer algorithms for identifying ORFs, such as GeneMark, are available and well known to those in the art. Comparison of the clone DNA to the complementary ORF (s) allows determination of whether the clone is a sense or antisense clone. Furthermore, each ORF extracted from the database can be compared to sequences in well annotated databases including the GenBank (nr) protein database, SWISSPROT and the like. A description of the gene or of a closely related gene in a closely related microorganism is often available in these databases. Similar methods are used to identify antisense clones corresponding to genes encoding non-translated RNAs.

In order to generate the gene identification data compiled in Table IB, each of the cloned nucleic acid sequences discussed above corresponding to SEQ ID NO. s 1-6213 was used to identify the corresponding Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa or Salrnonella typhinnzcoimn ORFs in the PathoSeq v. 4.1 (March 2000 release) database of microbial genomic sequences. For this purpose, the NCBI
BLASTN 2.0.9 computer algorithm was used. The default parameters were used except that filtering was turned off. The default parameters for the BLASTN and BLASTX analyses were:
Expectation value (e) =10
Alignment view options: pairwise
Filter query sequence (DUST with BLASTN, SEG with others) =T
Cost to open a gap (zero invokes behavior) =0
Cost to extend a gap (zero invokes behavior) =0
X dropoff value for gapped alignment (in bits) (zero invokes behavior) =0
Show GI's in deflines=F
Penalty for a nucleotide mismatch (BLASTN only) =! 3
Reward for a nucleotide match (BLASTN only) =l
Number of one-line descriptions (V) =500
Number of alignments to show (B) =250
Threshold for extending hits=default
Perform gapped alignment (not available with BLASTX) =T
Query Genetic code to use=l
DB Genetic code (for TBLAST [nx] only=l
Number of processors to use=l
SeqAlign file
Believe the query defline=F
Matrix=BLOSUM62
Word Size= default
Effective length of the database (use zero for the real size) =0

Number of best hits from a region to keep=100
Length of region used to judge hits=20
Effective length of the search space (use zero for the real size) =0
Query strands to search against database (for BLAST [nx] and TBLASTX), 3 is both, 1 is top, 2 is bottom=3
Produce HTML output=F
Alternatively, ORFs were identified and refined by conducting a survey of the public and private data sources. Full-length gene protein and nucleotide sequences for these organisms were assembled from various sources. For Pseudoinonas aeruginosa, gene sequences were adopted from the Pseudomonas genome sequencing project (downloaded from http://www. pseudomonas. com).

For Klebsiella pneumon. iae, Staphylococcus aureus, Streptococcus pfaemnoniae and Salrnonella typhi, genomic sequences from PathoSeq v 4.1 (Mar 2000 release) was reanalyzed for ORFs using the gene finding software GeneMark v 2.4a, which was purchased from GenePro Inc. 451 Bishop
St., N. W., Suite B, Atlanta, GA, 30318, USA.

Antisense clones were identified as those clones for which transcription from the inducible promoter would result in the expression of an RNA antisense to a complementary ORF, intergenic or intragenic sequence. Those clones containing single inserts and that caused growth sensitivity upon induction are listed in Table IA.

The gene descriptions in the PathoSeq database derive from annotations available in the public sequence databases described above. Where a clone was found to share significant sequence identity to two or more adjacent ORFs, it was listed once for each ORF and the PathoSeq information for each ORF was compiled in Table IB.

Table IA lists the SEQ ID NOs. and clone names of the inserts which inhibited proliferation. This information was used to identify the ORFs (SEQ ID NOs.: 6214-42397) whose gene products (SEQ ID NOs. 42398-78581) were inhibited by the nucleic acids comprising the nucleotide sequences of SEQ ID NOs. 1-6213. Table IB lists the clone name and the PathoSeq
Locus containing the clone.

TABLE IB
EMI244.1

EMI245.1

EMI246.1

EMI247.1

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EMI251.1

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EMI292.1

Table IC provides a cross reference between PathoSeq Gene Loci listed in Table IB and the
SEQ ID NOs. of the corresponding PathoSeq polypeptides and the SEQ ID NOs : of the nucleic acids which encode them. The Gene Locus IDs provided in Table IC each comprise a nine digit alpha-numeric identifier that can be used to determine the organism from which each Gene Locus and corresponding SEQ ID NOs. were identified. Specifically, the first letter of the Gene Locus ID corresponds to the first letter of the genus name of the organism described herein from which the
Gene Locus was identified and the second and third letters of the Gene Locus ID correspond to the first two letters of the species name of this organism. For example, the identifier EFA205257 describes a gene locus identified from Enterococcus faecalis. In those instances where the three letter identifier is the same for different organisms, the exact identity of the organism which corresponds to the Gene Locus ID can be determined by referring to the organism designation in the sequence listing for the coding nucleic acid or polypeptide SEQ ID NO. that corresponds to the particular Gene Locus ID.

TABLE IC
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EMI298.1

EMI299.1

EMI300.1

EMI301.1

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EMI513.1

It will be appreciated that ORFs may also be identified using databases other than PathoSeq. For example, the ORFs may be identified using the methods described in U. S.

Provisional Patent Application Serial Number 60/191,078, filed March 21,2000.

EXAMPLE 4

Transfer of Exogenous Nucleic Acid Sequences to other Bacterial Species

The ability of an antisense molecule identified in a first organism to inhibit the proliferation of a second organism (thereby confirming that a gene in the second organism which is homologous to the gene from the first organism is required for proliferation of the second organism) was validated using antisense nucleic acids which inhibit the growth of E. coli which were identified using methods similar to those described above. Expression vectors which inhibited growth of E. coli upon induction of antisense RNA expression with IPTG were transformed directly into Enterobacter cloacae, Klebsiella pneumonia or Salmonella typhimurium. The transformed cells were then assayed for growth inhibition according to the method of Example 1. After growth in liquid culture, cells were plated at various serial dilutions and a score determined by calculating the log difference in growth for INDUCED vs. UNINDUCED antisense RNA expression as determined by the maximum 10 fold dilution at which a colony was observed. The results of these experiments are listed below in Table II. If there was no effect of antisense RNA expression in a microorganism, the clone is minus in Table II. In contrast, a positive in Table II means that at least 10 fold more cells were required to observe a colony on the induced plate than on the non-induced plate under the conditions used and in that microorganism.

TABLE II

Sensitivity of Other Microorganisms to Antisense Nucleic Acids That Inhibit Proliferation in E. coli

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Thus, the ability of an antisense nucleic acid which inhibits the proliferation of Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella

pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium diffcile, Corynebacterium diptheriae, Enterobacter cloacae, Etzterococcus faeciuna, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylcoccus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis to inhibit the growth of other organims may be evaluated by transforming the antisense nucleic acid directly into species other than the organism from which they were obtained. In particular, the ability of the antisense nucleic acid to. inhibit the growth of Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioids immitis,
Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori,
Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella naultocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris,
Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori,
Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticu, Vibrio cholerae, Yabrio parahaernolyticus, Vabrio vulnaJacans, Yersinaa enterocolztzca, Yersinia pestis or any species falling within the genera of any of the above species may be evaluated. In some embodiments of the present invention, the ability of the antisense nucleic acid to inhibit the growth of an organism other than E. coli may be evaluated. In such embodiments, the antisense nucleic acids are inserted into expression vectors functional in the organisms in which the antisense nucleic acids are evaluated.

It will be appreciated that the above methods for evaluating the ability of an antisense nucleic acid to inhibit the proliferation of a heterologous organism may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from
Escherichia coli, Staplzylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertltssis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlarnydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile,
Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacteriuni tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,

Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including antisense nucleic acids complementary to SEQ ID NOs.: 6214-42397, such as the antisense nucleic acids of SEQ ID NOs.: 1-6213) or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids.

Those skilled in the art will appreciate that a negative result in a heterologous cell or microorganism does not mean that that cell or microorganism is missing that gene nor does it mean that the gene is unessential. However, a positive result means that the heterologous cell or microorganism contains a homologous gene which is required for proliferation of that cell or microorganism. The homologous gene may be obtained using the methods described herein. For example, the homologous gene may be isolated by performing a PCR procedure using primers based on the antisense sequence which reduced the level or activity of the gene product encoded by the homologous gene or by performing a Southern blot. Those cells that are inhibited by antisense may be used in cell-based assays as described herein for the identification and characterization of compounds in order to develop antibiotics effective in these cells or microorganisms. Those skilled in the art will appreciate that an antisense molecule which works in the microorganism from which it was obtained will not always work in a heterologous cell or microorganism.

EXAMPLES

Transfer of Exogenous Nucleic Acid Sequences to Other Bacterial Species Using the Escherichia coli.

Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa.

Salmonella tvphimurium. Acinetobacter baumannii. Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chamydia pneumoniae, Chlamydia trachomatic, Clostridium acetobutvlicum, Clostridium botulinum. Clostridium di ficile. Corynebacterium diphtheriae, Enterobcter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori. Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasina pneumoniae, Neisseria zollorrhoeae, Neisseria meningitidis, Pasteurella multocida, proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi,Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus mutans, Staphylococcus pneumoniae, Staphylococcus pyogenes, Treponema pallidum, Ureaplasma
urealvticum. Vibrio cholerae or Yersinia pestis Expression Vectors or Expression Vectors Functional in Bacterial Species Other Than the Foregoing Bacterial Species

The antisense nucleic acids that inhibit the growth of Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis,. Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemohilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculsis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis, or portions thereof, may also be evaluated for their ability to inhibit the growth of cells or microorganisms other than Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia} Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influefazae, Helicobacter pylori,

Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tubercuiosts, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae,
Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis. For example, the antisense nucleic acids that inhibit the growth of
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter
baumannii,
Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei,
Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatic, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloaceae, Enterococcus faecium,
Haemophilus inflaenzae, Helicobacter pylori, Legionella pneuntophila, Listeria monocytogenes,
Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella, multocida,
proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus
l1aemolyticus, Streptococcus mutans, Streptococcus pneumoniae,
Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or
Yersinia pestis may be evaluated for their ability to inhibit the growth of other organisms. In particular, the ability of the antisense nucleic acid to inhibit the
growth of Acinetobacter baumannii,
Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burklzolderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata), Candida tropicalis,
Candida parapsilosis, Candida guilliermondii, Candida krusei,
Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum,
Clostridium botulinum,
Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diphtheriae,
Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium,
Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebisella pheumoniae, Legionella pneumophila, Listeria
monocytogenes, Moraxella catarrhalis,
Mycobacterium avium, Mycobacteriunt bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasrna pneumoniae, Neisseria gonorrhoeae, Neisseria mertingitidis,
Nocardia asteroides, Pasteurella haemolytica, Pasteurella niultocida, Pneumocystis carinii,
Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis,
Salmonella enterica, Salmonella paratyphi,
Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, S'higellaflexneri,
Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans,
Streptococcus pyogenes, Treponema palZidum,
Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within
the genera of any of the above species may be evaluated. In some embodiments of the present invention, the ability of the antisense nucleic acid to inhibit the
growth of an organism other than E. coli may be evaluated.

In such methods, expression vectors in which the expression of an antisense nucleic acid that inhibits the growth of Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella
pertussis, Borrelia burgdorferi,

Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio choleraei or Yersinia pestisis under the control of an inducible promoter are introduced into the cells or microorganisms in which they are to be evaluated. In some embodiments, the antisense nucleic acids may be evaluated in cells or microorganisms which are closely related to Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrlialis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae,
Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis. The ability of these antisense nucleic acids to inhibit the growth of the related cells or microorganisms in the presence of the inducer is then measured.

EXAMPLE 6

Identification of Nucleic Acids Homologous to Nucleic Acids Required tor the iTohieration ot Staphvlococcus aureus in other Bacterial Species

Nucleic acids homologous to proliferation-required nucleic acids from Staphylococcus aureus were identified as follows. For example, thirty-nine antisense nucleic acids which inhibited the growth of Staphylococcus aureus were identified using methods such as those described herein and were inserted into an expression vector such that their expression was under the control of a xyloseinducible Xyl-T5 promoter. A vector with a reporter gene under control of the Xyl-T5 promoter was. used to show that expression from the Xyl-T5 promoter in Staphylococcus epidermidis was comparable to that in Staphylococcus aureus.

The vectors were introduced into Staphylococcus epidermidis by electroporation as follows:
Staphylococcus epidermidis was grown in liquid culture to mid-log phase and then harvested by centrifugation. The cell pellet was resuspended in 1/3 culture volume of ice-cold EP buffer (0.625
M sucrose, 1 mM MgCl2, pH=4.0), and then harvested again by centrifugation. The cell pellet was then resuspended with 1/40 volume EP buffer and allowed to incubate on ice for 1 hour. The cells were then frozen for storage at-80 C. For electroporation, 50 lil of thawed electrocompetent cells were combined with 0.5 ptg plasmid DNA and then subjected to an electrical pulse of 10 kV/cm, 25 uFarads, 200 ohm using a biorad gene pulser electroporation device. The cells were immediately resuspended with 200 u. l outgrowth medium and incubated for 2 hours prior to plating on solid growth medium with drug selection to maintain the plasmid vector. Colonies resulting from overnight growth of these platings were selected, cultured in liquid medium with drug selection, and then subjected to dilution plating analysis as described for Staphylococcus aureus in Example 1 above to test growth sensitivity in the presence of the inducer xylose.

The results are shown in Table III below. The first column indicates the Molecule Number of the Staphylococcus aureus antisense nucleic acid which was introduced into Staphylococcus epidermidis. The second column indicates whether the antisense nucleic acid inhibited the growth of Staphylococcus epidermidis, with a"+"indicating that growth was inhibited. Of the 39
Staphylococcus aureus antisense nucleic acids evaluated, 20 inhibited the growth of Staphylococcus epidermidis..

TABLE III

Sensitivity of Other Microorganisms to Antisense Nucleic Acids That Inhibit Proliferation of Staphylococcus aureus

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EMI524.1

Although the results shown above were obtained using a suset o'proiifer'ationlrelitred nucleic acids from Staphylococcus aureus, it will be appreciated that similar analyses may be performed using the nucleic acids of the present invention to determine whether they inhibit the proliferation of cells or microorganisms other than Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsilla pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium,
Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlanaydia trachonaatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptlieriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meni77gitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epiderrnidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis,
Thus, it will be appreciated that the above methods for evaluating the ability of an antisense nucleic acid to inhibit the proliferation of a heterologous organism may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridum difficile, corynebacterium diphtheriae, Enterobacer cloacae, Enterococcus faerium Haemophilus influerazae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis, (including antisense nucleic acids complementary to SEQ ID NOs.: 6214-42397, such as the antisense nucleic acids of SEQ ID NOs.: 1-6213) or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids.

EXAMPLE 7

Identification of Homologous Nucleic Acids by Functional Complementation

Homologous coding nucleic acids, homologous antisense nucleic acids or nucleic acids encoding homologous polypeptides may be identified as follows. Gene products whose activities may be complemented by a proliferation-required gene product from Escherichia coli,
Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa,
Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis,
Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,

Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidef rnidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Yersinia pestis or homologous polypeptides are identified using merodiploids, created by introducing a plasmid or Bacterial Artificial Chromosome into an organism having a mutation in the essential gene which reduces or eliminates the activity of the gene product. In some embodiments, the mutation may be a conditional mutation, such as a temperature sensitive mutation, such that the organism proliferates under permissive conditions but is unable to proliferate under non-permissive conditions in the absence of complementation by the gene on the plasmid or Bacterial Artificial Chromosome. Alternatively, duplications may be constructed as described in Roth et al. (1987) Biosynthesis of Aromatic Amino Acids in Escherichia coli and Salmonella typhimurium, F. C. Neidhardt, ed., American Society for Microbiology, publisher, pp. 2269-2270. Such methods are familiar to those skilled in the art.

Alternatively, homologous coding nucleic acids, homologous antisense nucleic acids or nucleic acids encoding homologous polypeptides may be identified by placing a gene required for proliferation or a nucleic acid complementary to at least a portion of a gene required for proliferation under the control of a regulatable promoter as described above, introducing a plasmid or Bacterial Artificial Chromosome into the cell, and identifying cells which are able to proliferate under conditions which would prevent or reduce proliferation in the absence of the plasmid or Bacterial Artificial Chromosome.

Homologous coding nucleic acids, homologous antisense nucleic acids or nucleic acids encoding homologous polypeptides may be identified using databases as follows.

EXAMPLE 8---
Identification of Homologous Nucleic Acids by Database Analysis
As a demonstration of the methodology required to find homologues to an essential gene, fifty-one prokaryotic organisms were analyzed and compared in detail. First, the most reliable source of gene sequences for each organism was assessed by conducting a survey of the public and private data sources. The fifty-one organisms studied are Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimuriurn, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae and Yersinia pestis. Full-length gene protein and nucleotide sequences for these organisms were assembled from various sources. For Escherichia coli, Haemophilus influenzae and Helicobacter pylori, gene sequences were adopted from the public sequencing projects, and derived from the GenPept 115 database (available from NCBI). For Pseudomonas aeruginosa, gene sequences were adopted from the Pseudomonas genome sequencing project (downloaded from http ://www. pseudomonas. com). For Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae and Salmonella typhi, genomic sequences from PathoSeq v 4.1 (Mar 2000 release) were reanalyzed for ORFs using the gene finding software GeneMark v 2.4a, which was purchased from GenePro Inc. 451 Bishop St., N. W., Suite B, Atlanta, GA, 30318, USA. Similar analyses were conducted for the other organisms using publically available and proprietary databases.

Homologous coding nucleic acids and the homologous polypeptides which they encode may be identified using a"reciprocal"best-hit analysis. To facilitate the identification of homologous coding nucleic acids and homologous polypeptides, paralogous genes within each of 51 organisms were identified and

clustered prior to comparison to other organisms. Briefly, the polypeptide sequence of each polypeptide encoded by each open reading frame (ORF) in a given organism was compared to the polypeptide sequence encoded by every other ORF for that organism for each of the 51 pathogenic organisms (PathoSeq Sept 2001 release) using BLASTP 2.09 algorithm without filtering. Simultaneously, the polypeptide sequence encoded by each ORF of an organism was compared to the polypeptide sequences encoded by each of the ORFs in the remaining 51 organisms. Those polypeptides within a single organism that shared a higher degree of sequence identity to one another than to polypeptide sequences obtained from any other organisms were clustered as"paralog"sequences for"reciprocal"best-hit analysis.

For each reference organism, the 50 homologous coding nucleic acids (and the 50 homologous polypeptides which they encode) was determined by identifying the ORFs in each of the 50 comparison organisms which encode a polypeptide sharing the highest degree of amino acid sequence identity to the polypeptide encoded by the ORF from the reference organism. The accuracy of the identification of the predicted homologous coding nucleic acids (and the homologous polypeptides which they encode) was confirmed by a"reciprocal"BLAST analysis in which the polypeptide sequence of the predicted homologous polypeptide was compared against the polypeptides encoded by each of the ORFS in the reference organism using BLASTP 2.09 algorithm without filtering. Only those polypeptides that share the highest degree of amino acid sequence identity in each portion of the two-way comparison are retained for further analysis.

The best homolog for each of the fifty-one organisms, defined as the most significantly scoring match which also fulfilled the above criteria, was reported in Table IV.

Table IV lists the best ORF identified as described above (column labeled Homolog
LocusID) that matches the query sequence (column labeled Query LocusID), % identity between the query sequence and the homolog, and the amount of each sequence that aligns together well (columns labeled Query Coverage and Homolog Coverage) for the gene identified in each of the fifty-one organisms evaluated as described above. As described in connection which Table IC, the
Locus IDs (ie. both Query Locus ID and Homolog Locus ID) provided in Table IV each comprise a nine digit alpha-numeric identifier that can be used to determine the organism from which the query and homolog sequences were obtained. Specifically, the first letter of the Locus ID corresponds to the first letter of the genus name of the organism described herein from which the Locus was identified and the second and third letters of the Locus ID correspond to the first two letters of the species name of this organism. For example, the identifier EFA205257 describes a gene locus identified from Etiterococcus faecalis. In those instances where the three letter identifier is the same for different organisms, the exact identity of the organism which corresponds to the Locus ID can be determined by referring to the organism designation in the sequence listing for the coding nucleic acid or polypeptide SEQ ID NO. that corresponds to the particular Locus ID.

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The data in Table IV demonstrates the methods described herein identified genes required for proliferation in several species which share homology.

EXAMPLE 9

Identification of Genes and their Corresponding Operons Affected by Antisense Inhibition

Once the genes involved in Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aerugifaosa, Salmonella typhifnuriurn, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burklaolderia cepacia, Burkholderia fungorufn, Burlcholderia mallei, Campylobacter jejuni,

Chlamydia pneumoniae, Chlamydia traclaofnatis, Clostridium acetobutylicurn, Clostridium botulinum, Clostridium dcile, Coryfaebacteriurn diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis proliferation are identified as described above, the operons in which these genes lie may be identified by comparison with known microbial genomes. Since bacterial genes are transcribed in a polycistronic manner, the antisense inhibition of a single gene in an operon might affect the expression of all the other genes on the operon or the genes downstream from the single gene identified. Accordingly, each of the genes contained within an operon may be analyzed for their effect on proliferation.

Operons are predicted by looking for all adjacent genes in a genomic region that lie in the same orientation with no large noncoding gaps in between. First, full-length ORFs complementary to the antisense molecules are identified as described above. Adjacent ORFs are then identified and their relative orientation determined either by directly analyzing the genomic sequences surrounding the ORFs complementary to the antisense clones or by extracting adjacent ORFs from the collection obtained through whole genome ORF analysis described above followed by ORF alignment. Operons predicted in this way may be confirmed by comparison to the arrangement of the homologous nucleic acids in the Bacillus subtilis complete genome sequence, as reported by the genome database compiled at Institut Pasteur Subtilist Release R15. 1 (June 24,1999) which can be found at http ://bioweb. pasteur. fr/GenoList/SubtiList/. The Bacillus subtilis genome is the only fully sequenced and annotated genome from a Gram positive microorganism, and appears to have a high level of similarity to Staphylococcus aureus both at the level of conservation of gene sequence and genomic organization including operon structure. Operons for Salmonella typhimurium and
Klebsiella pneumoniae may be identified by comparison with E. coli, Haemophilus, or
Pseudomonas sequences. The Pseudomonas aeruginosa web site (http://www. pseudomonas. com) can also be used to help predict operon organization in this bacterium.

Extensive DNA sequences of Salmonella typhimurium are available through the Salmonella
Genome Center (Washington University, St. Louis, MO) the Sanger Centre (United Kingdom) and the PathoSeq database (Incyte). Annotation of some of the DNA sequences in some of the aforementioned databases is lacking, but comparisons may be made to E. coli using tools such as
BLASTX.

Public or proprietary databases may be used to analyzed E. faecalis sequences as well as sequences from the organisms listed above.

The analysis of the operons on which essential genes lie may be conducted for each of the sequences which are listed in Table IA which inhibit proliferation and the ORFs listed in Table IC.

Once the full length ORFs and/or the operons containing them have been identified using the methods described above, they can be obtained from a genomic library by performing a PCR amplification using primers at each end of the desired sequence. Those skilled in the art will appreciate that a comparison of the ORFs to homologous sequences in other cells or microorganisms will facilitate confirmation of the start and stop codons at the ends of the ORFs.

In some embodiments, the primers may contain restriction sites which facilitate the insertion of the gene or operon into a desired vector. For example, the gene may be inserted into an expression vector and used to produce the proliferation-required protein as described below. Other methods for obtaining the full length ORFs and/or operons are familiar to those skilled in the art.

For exmaple, natural restriction sites may be employed to insert the full length ORFs and/or operons into a desired vector.

EXAMPLE 10

Identification of Individual Genes within an Operon Required for Proliferation

The following example illustrates a method for determining if a targeted gene within an operon is required for cell proliferation by replacing the targeted allele in the chromosome with an in-frame deletion of the coding region of the targeted gene.

Deletion inactivation of a chromosomal copy of a gene in Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinunt, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrh lis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis can be accomplished by integrative gene replacement. The principles of this method were described in Xia, M., et al. 1999 Plasmid 42: 144 149 and Hamilton, C. M., et al 1989. J. Bacteriol. 171: 4617-4622. A similar gene disruption method is available for Pseudomonas aeruginosa, except the counter selectable marker is sacB (Schweizer, H. P., Klassen, T. and Hoang, T. (1996) Mol. Biol. of Pseudomonas. ASM press, 229237. In this approach, a mutant allele of the targeted gene is constructed by way of an in-frame deletion and introduced into the chromosome using a suicide vector. This results in a tandem duplication comprising a deleted (null) allele and a wild type allele of the target gene. Cells in which the vector sequences have been deleted are isolated using a counter-selection technique.

Removal of the vector sequence from the chromosomal insertion results in either restoration of the wild-type target sequence or replacement of the wild type sequence with the deletion (null) allele.

E. faecalis genes can be disrupted using a suicide vector that contains an internal fragment to a gene of interest. With the appropriate selection this plasmid will homologously recombine into the chromosome (Nallapareddy, S. R., X. Qin, G. M. Weinstock, M. Hook, B. E. Murray. 2000. Infect. Immun. 68: 5218-5224.

The resultant population of Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium digicile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis colonies can then be evaluated to determine whether the target sequence is required for proliferation by PCR amplification of the affected target sequence. If the targeted gene is not required for proliferation, then PCR analysis will show that roughly equal numbers of colonies have retained either the wild-type or the mutant allele. If the targeted gene is required for proliferation, then only wild-type alleles will be recovered in the PCR analysis.

The method of cross-over PCR is used to generate the mutant allele by amplification of nucleotide sequences flanking but not including the coding region of the gene of interest, using specifically designed primers such that overlap between the resulting two PCR amplification products allows them to hybridize. Further PCR amplification of this hybridization product using primers representing the extreme 5'and 3'ends can produce an amplification product containing an in-frame deletion of the coding region but retaining substantial flanking sequences.

For Staphylococcus aureus, this amplification product is subcloned into the suicide vector pSA3182 (Xia, M., et al. 1999 Plasmid 42: 144-149, which is host-dependent for autonomous replication. This vector includes a tetC tetracycline-resistance marker and the origin of replication of the well-known Staphylococcus aureus plasmid pT181 (Mojumdar, M and Kahn, S. A., Characterisation of the Tetracycline Resistance Gene of Plasmid pT181, J. Bacteriol. 170: 5522 (1988). The vector lacks the repC gene which is required for autonomous replication of the vector at the pT181 origin. This vector can be propagated in a Staphylococcus aureus host strain such as SA3528, which expresses repC in trans. Once the amplified truncated target gene sequence is cloned and propagated in the pSA3182 vector, it can then be introduced into a repC minus strain such as RN4220 (Kreiswirth, B. N. et al., The Toxic Shock Syndrome Exotoxin Structural Gene is Not Detectably Transmitted by a Prophage, Nature 305: 709-712 (1983), by electroporation with selection for tetracycline resistance. In this strain, the vector must integrate by homologous recombination at the targeted gene in the chromosome to impart drug resistance. This results in a inserted truncated copy of the allele, followed by pSA3182 vector sequence, and finally an intact and functional allele of the targeted gene.

Once a tetracycline resistant Staphylococcus aureus strain is isolated using the above technique and shown to include truncated and wild-type alleles of the targeted gene as described above, a second plasmid, pSA7592 (Xia, M., et al. 1999 Plasmid 42: 144-149, is introduced into the strain by electroporation. This gene includes an erythromycin resistance gene and a repC gene that is expressed at high levels. Expression of repC in these transformants is toxic due to interference of normal chromosomal replication at the integrated pT181 origin of replication. This selects for strains that have removed the vector sequence by homologous recombination, resulting in either of two outcomes: The selected cells either possess a wild-type allele of the targeted gene or a gene in which the wild-type allele has been replaced by the engineered in-frame deletion of the truncated allele.

* PCR amplification can be used to determine the genetic outcome of the above process in the resulting erythromycin resistant, tet sensitive transformant colonies. If the targeted gene is not required for cellular replication, then PCR evidence for both wild-type and mutant alleles will be found among the population of resultant transformants. However, if the targeted gene is required for cellular proliferation, then only the wild-type form of the gene will be evident among the resulting transformants.

Similarly, for Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorurn, Burklzolderia mallei, Carnpylobacter jejuni, Chlatnydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealtyicum, Vibrio cholerae or Yersinia pestis the PCR products containing the mutant allele of the target sequence may be introduced into an appropriate knockout vector and cells in which the wild type target has been disrupted are selected using the appropriate methodology.

The above methods have the advantage that insertion of an in-frame deletion mutation is far less likely to cause downstream polar effects on genes in the same operon as the targeted gene.

However, it will be appreciated that other methods for disrupting Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhirnurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faeciuna, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberCulosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Urealpasam urealyticum, Vibrio cholerae or Yersinia pestis genes which are familiar to those skilled in the art may also be used.

Each gene in the operon may be disrupted using the methodology above to determine whether it is required for proliferation.

EXAMPLE 11
Expression of the Proteins Encoded by Genes Identified as Required for Escherichia coli,
Staphvlococcus aureus, Eriterococcus faecalis. Klebsiella pneumoniae, Pseudomonas aeruginosa.

Salmonella tvphimurium, Acinetobacter baumannSi, Bacillus anthracis, Bacteroides fragilis,
Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, BurkAlolderiamallei, CampvlobacterjeSuni,
Chlamvdiapneumoniae, Chlamvdiatrachomatis,
Clostridium acetobutylicum. Clostridium botulinum, Clostridium difficile. Conebacterium diptheriae, Enterobacter cloacae Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium ycobacterium tuberculosis, M co ma jzenitalium, M ia jzonorrhoeae, Neisseria meninjzitidis, Pasteurella multocida. Proteus mirabilis. Pseudomonas putida, Pseudomonas syringae, Salmonella paratvphi.

Salmonella tvphi. Staphylococcus epidermidis. Staphlococcus haemolvticus. Streptococcus
Streptococcus pneumon Lponema pallidum, Ureqplasma urealyticum Vibrio cholerae. Yersinia pestis Proliferation
The following is provided as one exemplary method to express the proliferation-required proteins idenfied as described above. The proliferation-required proteins may be expressed using any of the bacterial, insect, yeast, or mammalian expression systems known in the art. In some embodiments, the proliferation-required proteins encoded by the identified nucleotide sequences described above (including the proteins of SEQ ID NOs.: 42398-78581 encoded by the nucleic acids of
SEQ ID NOs.: 6214-42397 are expressed using expression systems designed either for E. coli or for
Staphylococcus aureur, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa,
Salmonella typhifnuriusn, Acinetobacter bamnannii) Bacillus anthracis, Bacteroides fragilis,
Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicurn, Clostridium botulinum, Clostridiurn dcile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis. First, the initiation and termination codons for the gene are identified. If desired, methods for improving translation or expression of the protein are well known in the art. For example, if the nucleic acid encoding the polypeptide to be expressed lacks a

methionine codon to serve as the initiation site, a strong Shine-Delgarno sequence, or a stop codon, these nucleotide sequences can be added. Similarly, if the identified nucleic acid lacks a transcription termination signal, this nucleotide sequence can be added to the construct by, for example, splicing out such a sequence from an appropriate donor sequence. In addition, the coding sequence may be operably linked to a strong constitutive promoter or an inducible promoter if desired. The identified nucleic acid or portion thereof encoding the polypeptide to be expressed is obtained by, for example,
PCR from the bacterial expression vector or genome using oligonucleotide primers complementary to the identified nucleic acid or portion thereof and containing restriction endonuclease sequences appropriate for inserting the coding sequences into the vector such that the coding sequences can be expressed from the vector's promoter. Alternatively, other conventional cloning techniques may be used to place the coding sequence under the control of the promoter. In some embodiments, a termination signal may be located downstream of the coding sequence such that transcription of the coding sequence ends at an appropriate position.

Several expression vector systems for protein expression in E. coli are well known and available to those knowledgeable in the art. The coding sequence may be inserted into any of these vectors and placed under the control of the promoter. The expression vector may then be transformed into DH5a or some other E. coli strain suitable for the over expression of proteins.

Alternatively, an expression vector encoding a protein required for proliferation of
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae,
Chlafnydia trachomatis, Clostridium acetobutylicum, Clostridium botulitaum, Clostridium difficile,
Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis may be introduced into Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii,
Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burklaolderia fungorum, Burkholderia mallei, Campylobacter jejuni, Clalamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, corynebacterium diptheriae, Enterobacter cloacae, enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes,
Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae,
Streptococcus pyogenes, Treponema pallidutn, Ureaplasma urealyticuna, Vibrio cholerae or
Yersinia pestis. Protocols for introducing nucleic acids into these organisms are well known in the art. For example, the protocols described in J. C. Lee"Electroporation of Staphylococci"from
Methods in Molecular Biology vol 47: Electroporation Protocols for Microorganisms Edited by:
J. A. Nickoloff Humana Press Inc., Totowa, NJ. pp209-216, may be used to introduce nucleic acids into StapZlylococcus aureus. Nucleic acids may also be introduced into Salmonella typhimurium,
Klebsiella pneumoniae, Pseudomonas aeruginosa or Enterococcus faecalis using methods familiar to those skilled in the art. Positive transformants are selected after growing the transformed cells on plates containing an antibiotic to which the vector confers resistance. In one embodiment, StapStylococcus

aureus is transformed with an expression vector in which the coding sequence is operably linked to the T5 promoter containing a xylose operator such that expression of the encoded protein is inducible with xylose.

In one embodiment, the protein is expressed and maintained in the cytoplasm as the native sequence. In an alternate embodiment, the expressed protein can be modified to include a protein tag that allows for differential cellular targeting, such as to the periplasmic space of Gram negative or Gram positive expression hosts or to the exterior of the cell (i. e., into the culture medium). In some embodiments, the osmotic shock cell lysis method described in Chapter 16 of Current
Protocols in Molecular Biology, Vol. 2, (Ausubel, et al., Eds.) John Wiley & Sons, Inc. (1997) may be used to liberate the polypeptide from the cell. In still another embodiment, such a protein tag could also facilitate purification of the protein from either fractionated cells or from the culture medium by affinity chromatography. Each of these procedures can be used to express a proliferationrequired protein.

Expressed proteins, whether in the culture medium or liberated from the periplasmic space or the cytoplasm, are then purified or enriched from the supernatant using conventional techniques such as ammonium sulfate precipitation, standard chromatography, immunoprecipitation, immunochromatography, size exclusion chromatography, ion exchange chromatography, and HPLC.

Alternatively, the polypeptide may be secreted from the host cell in a sufficiently enriched or pure state in the supernatant or growth media of the host cell to permit it to be used for its intended purpose without further enrichment. The purity of the protein product obtained can be assessed using techniques such as SDS PAGE, which is a protein resolving technique well known to those skilled in the art. Coomassie, silver staining or staining with an antibody are typical methods used to visualize the protein of interest.

Antibodies capable of specifically recognizing the protein of interest can be generated using synthetic peptides using methods well known in the art. See, Antibodies: A Laboratory Manual, (Harlow and Lane, Eds.) Cold Spring Harbor Laboratory (1988). For example, 15-mer peptides having an amino acid sequence encoded by the appropriate identified gene sequence of interest or portion thereof can be chemically synthesized. The synthetic peptides are injected into mice to generate antibodies to the polypeptide encoded by the identified nucleic acid sequence of interest or portion thereof. Alternatively, samples of the protein expressed from the expression vectors discussed above can be purified and subjected to amino acid sequencing analysis to confirm the identity of the recombinantly expressed protein and subsequently used to raise antibodies. An Example describing in detail the generation of monoclonal and polyclonal antibodies appears in Example 12.

The protein encoded by the identified nucleic acid of interest or portion thereof can be purified using standard immunochromatography techniques. In such procedures, a solution containing the secreted protein, such as the culture medium or a cell extract, is applied to a column having antibodies against the secreted protein attached to the chromatography matrix. The secreted protein is allowed to bind the immunochromatography column. Thereafter, the column is washed to remove nonspecifically bound proteins. The specifically-bound secreted protein is then released from the column and recovered using standard techniques. These procedures are well known in the art.

In an alternative protein purification scheme, the identified nucleic acid of interest or portion thereof can be incorporated into expression vectors designed for use in purification schemes employing chimeric polypeptides. In such strategies the coding sequence of the identified nucleic acid of interest or portion thereof is inserted in-frame with the gene encoding the other half of the chimera. The other half of the chimera can be maltose binding protein (MBP) or a nickel binding polypeptide encoding sequence. A chromatography matrix having maltose or nickel attached thereto is then used to purify the chimeric protein. Protease cleavage sites can be engineered between the MBP gene or the nickel binding polypeptide and the identified expected gene of interest, or portion thereof. Thus, the two polypeptides of the chimera can be separated from one another by protease digestion.

One useful expression vector for generating maltose binding protein fusion proteins is pMAL (New England Biolabs), which encodes the malE gene. In the pMal protein fusion system, the cloned gene is inserted into a pMal vector downstream from the malE gene. This results in the expression of an MBP-fusion protein. The fusion protein is purified by affinity chromatography. These techniques as described are well known to those skilled in the art of molecular

biology.

EXAMPLE 12

Production of an Antibody to an isolated Escherichia coli, Staphylococcus aureus. Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella tvphimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia forum. Burkholderia mallei. Campvlobacter ejuni, Chlamvdia pneumoniae, Chlamvdia trachomatis, Clostridium, acetobutvlicum, Clostridium botulinum, Clostridium difficile, Coanebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pvlori, Leionella pneumophila. Listeria inonocytoze ycobacteriuln bovis, Mvcobacterium leprae. Mvcobacterium tuberculosis. Mcoplasma genitalium. Mvcoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratvplti, Salmonella tvphi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus mutans, Staphylococcus pneumoniae, Staphylococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis Protein

Substantially pure protein or polypeptide (including one of the polypeptides of SEQ ID NOs.: 42398-78581) is isolated from the transformed cells as described in Example 11. The concentration of protein in the final preparation is adjusted, for example, by concentration on a 10,000 molecular weight cut off AMICON filter device (Millipore, Bedford, MA), to the level of a few micrograms/ml.

Monoclonal or polyclonal antibody to the protein can then be prepared as follows:

Monoclonal Antibody Production by Hybridoma Fusion

Monoclonal antibody to epitopes of any of the peptides identified and isolated as described can be prepared from murine hybridomas according to the classical method of Kohler, G. and Milstein, C., Nature 256: 495 (1975) or any of the well-known derivative methods thereof. Briefly, a mouse is repetitively inoculated with a few micrograms of the selected protein or peptides derived therefrom over a period of a few weeks. The mouse is then sacrificed, and the antibody-producing cells of the spleen isolated. The spleen cells are fused by means of polyethylene glycol with mouse myeloma cells, and the excess unfused cells are destroyed by growth of the system on selective medium comprising aminopterin (HAT medium). The successfully-fused cells are diluted and aliquots of the dilution placed in wells of a microtiter plate where growth of the culture is continued. Antibody-producing clones are identified by detection of antibody in the supernatant fluid of the wells by immunoassay procedures, such as ELISA, as described by Engvall, E.,"Enzyme immunoassay ELISA and EMIT," Meth. Enzymol. 70: 419 (1980), and derivative methods thereof. Selected positive clones can be expanded and their monoclonal antibody product harvested for use. Detailed procedures for monoclonal antibody production are described in Davis, L. et al. Basic Methods in Molecular Biology Elsevier, New York. Section 21-2.

Polyclonal Antibody Production by Immunization

Polyclonal antiserum containing antibodies to heterogeneous epitopes of a single protein or a peptide can be prepared by immunizing suitable animals with the expressed protein or peptides derived therefrom described above, which can be unmodified or modified to enhance immunogenicity.

Effective polyclonal antibody production is affected by many factors related both to the antigen and the host species. For example, small molecules tend to be less immunogenic than larger molecules and can require the use of carriers and adjuvant. Also, host animals vary in response to site of inoculations and dose, with both inadequate or excessive doses of antigen resulting in low titer antisera. Small doses (ng level) of antigen administered at multiple intradermal sites appears to be most reliable. An effective immunization protocol for rabbits can be found in Vaitukaitis, J. et al. J. Clin. Endocrinol. Metab. 33: 988-991 (1971).

Booster injections can be given at regular intervals, and antiserum harvested when antibody titer thereof, as determined semi-quantitatively, for example, by double immunodiffusion in agar against known concentrations of the antigen, begins to fall. See, for example, Ouchterlony, O. et al., Chap. 19 in: Handbook of Experimental Immunology D. Wier (ed) Blackwell (1973). Plateau concentration of antibody is usually in the range of 0.1 to 0.2

mg/ml of serum (about 12 1). Affinity of the antisera for the antigen is determined by preparing competitive binding curves, as described, for example, by Fisher, D., Chap. 42 in: Manual of Clinical Immunology, 2d Ed. (Rose and Friedman, Eds.) Amer. Soc. For Microbiol., Washington, D. C. (1980).

Antibody preparations prepared according to either protocol are useful in quantitative immunoassays which determine concentrations of antigen-bearing substances in biological samples; they are also used semi-quantitatively or qualitatively to identify the presence of antigen in a biological sample. The antibodies can also be used in therapeutic compositions for killing bacterial cells expressing the protein.

EXAMPLE 13

Construction of Strains Which Overexpress or Underexpress Gene Products Required for Proliferation by Promoter Replacement

Strains which overexpress or underexpress gene products required for proliferation may also be constructed by replacing the promoters which naturally direct transcription of these gene products with promoters which provide the desired level of expression. As described above, such strains are useful in methods for identifying essential genes, in methods for identifying compounds which inhibit cellular proliferation, in methods for identifying the targets of compounds which inhibit proliferation, as well as in methods for identifying genes encoding gene products required for proliferation. Some embodiments of the present invention contemplate the use of a vector that comprises a regulatable fusion promoter selected from a suite of fusion promoters wherein the promoter suite is useful for modulating both the basal and maximal levels of transcription of a nucleic acid over a wide dynamic range thus allowing the desired level of production of a transcript which corresponds to a nucleic acid described herein. Such promoters are described in U. S. Patent Application Serial Number 10/032,393, filed December 21,2001, the disclosure of which is incorported herein by reference in its entirety.

For example, in some embodiments, the natural promoter may be replaced using techniques which employ homologous recombination to exchange a promoter present on the chromosome of the cell with the desired promoter. In such methodology, a nucleic acid comprising a promoter replacement cassette is introduced into the cell. As illustrated in Figure 1A, the promoter replacement cassette comprises a 5'region homologous to the sequence which is 5'of the natural promoter in the chromosome, the promoter which is to replace the chromosomal promoter and a 3' region which is homologous to sequences 3'of the natural promoter in the chromosome. In some embodiments, the promoter replacement cassette may also include a nucleic acid encoding an identifiable or selectable marker disposed between the 5'region which is homologous to the sequence 5'of the natural promoter and the promoter which is to replace the chromosomal promoter. If desired, the promoter replacement cassette may also contain a transcriptional terminator 3'of the gene encoding an identifiable or selectable marker, as illustrated in Figure 1B.

As illustrated in Figure 1A and 1B, homologous recombination is allowed to occur between the chromosomal region containing the natural promoter and the promoter replacement cassette. Cells in which the promoter replacement cassette has integrated into the chromosome are identified or selected. To confirm that homologous recombination has occurred, the chromosomal structure of the cells may be verified by Southern analysis or PCR.

In some embodiments, the promoter replacement cassette may be introduced into the cell as a linear nucleic acid, such a PCR product or a restriction fragment. Alternatively, the promoter replacement may be introduced into the cell on a plasmid. Figures 1A and 1B illustrates the replacement of a chromosomal promoter with a desired promoter through homologous recombination.

In some embodiments, the cell into which the promoter replacement cassette is introduced may carry mutations which enhance its ability to be transformed with linear DNA or which enhance the frequency of homologous recombination. For example, if the cell is an Escherichia coli cell it may have a mutation in the gene encoding Exonuclease V of the RecBCD recombination complex.

If the cell is an Escherichia coli cell it may have a mutation that activates the RecET recombinase of the Rac prophage and/or a mutation that enhances recombination through the RecF pathway. For example, the Escherichia coli cells may be RecB or RecC mutants carrying an sbcA or sbcB mutation. Alternatively, the Escherichia coli cells may be recD mutants. In other embodiments the

Escherichia coli cells may express the k Red recombination genes. For example, Escherichia coli cells suitable for use in techniques employing homologous recombination have been described in
Datsenko, K. A. and Warmer, B. L., PNAS 97: 6640-6645 (2000); Murphy, K. C., J. Bact 180: 20532071 (1998); Zhang, Y., et al., Nature Genetics 20: 123-128 (1998); and Muyrers, J. P. P. et al.,
Genes & Development 14: 1971-1982 (2000). It will be appreciated that cells carrying mutations in similar genes may be constructed in organisms other than Escherichia coli.

In some embodiments, the methods described in U. S. Patent Application Serial Number 09/948,993, may be used to place the gene required for proliferation under the control of a regulatable promoter.

If the organism in which promoter replacement is to be performed is diploid, strains in which genes encoding gene products required for proliferation are under the control of a desired promoter may be constructed by inactivating one chromosomal copy of a gene encoding a gene product required for proliferation. For example, the gene may be inactivated by insertion of or replacement by a nucleotide sequence encoding a selectable or detectable gene product, such as a polypeptide which provides resistance to a drug or which allows growth under certain culture conditions. The other chromosomal copy of the gene encoding a gene product required for proliferation is placed under the control of a regulatable promoter, such as the tetracycline regulatable promoter similar to that described in Gari et al., (1997) Yeast 13: 837-848 and Nagahashi et al., (1997) Mol. Gen. Genet. 255: 372-375, by homologous recombination. The resultant strains may be used to identify genes which encode gene products required for proliferation and in the methods of the present invention.

The method may also be applied to haploid organisms by modifying the single allele of the gene via recombination of the allele with a promoter replacement fragment comprising a nucleotide sequence encoding a heterologous promoter, such that the expression of the gene is conditionally regulated by the heterologous promoter. By repeating this process for a preferred subset of genes in a haploid pathogenic organism, or its entire genome, a collection or a complete set of conditional mutant strains can be obtained.

It will be appreciated that the means to achieve conditional expression are not restricted to the promoters discussed above and can be performed with other conditional promoters. Such conditional promoter may, for example, be regulated by a repressor which repress transcription from the promoter under particular condition or by a transactivator which increases transcription from the promoter, such as, when in the presence of an inducer.

Although not mandatory, performing the gene disruption first enables heterozygous strains to be constructed and separately collected as a heterozygote strain collection during the process of drug target validation. Heterozygous strains for a given gene express approximately half the normal diploid level of a particular gene product. Consequently, these strains provide constructions having a diminished level of the encoded gene product, and they may be used in the methods described herein. However, it is clear to those skilled in the art that the order of allele modification followed in this embodiment of the invention is not critical, and that it is feasible to perform these steps in a different order such that the conditional-expressing allele is constructed first and the disruption of the remaining wild type gene allele be performed subsequently.

However, where the promoter replacement step is carried out first, it is preferable to delete sequences homologous to those employed in the gene disruption step.

Alternatively, conditional expression could be achieved by means other than the reliance of conditional promoters. For example, conditional expression could be achieved by the replacement of the wild type allele in haploid or heterozygous strains with temperature sensitive alleles derived in vitro, and their phenotype would then be analyzed at the nonpermissive temperature. In a related approach, in heterozygous strains, insertion of a ubiquitination signal into the remaining wild type allele to destabilize the gene product during activation conditions can be adopted to examine phenotypic effects resulting from gene inactivation.

In another alternative, a constitutive promoter regulated by an excisable transactivator can be used. The promoter is placed upstream to a target gene to

repress expression to the basal level characteristic of the promoter. For example, if the strains are fungal organisms, a heterologous promoter containing lexA operator elements may be used in combination with a fusion protein composed of the lexA DNA binding domain and any transcriptional activator domain (e. g. GAL4,
HAP4, VP16) to provide constitutive expression of a target gene. Counterselection mediated by 5
FOA can be used to select those cells which have excised the gene encoding the fusion protein.

This procedure enables an examination of the phenotype associated with repression of the target gene to the basal level of expression provided by the lexA heterologous promoter in the absence of a functional transcription activator. The strains generated by this approach may be used in the present invention.

Alternatively, conditional expression of a target gene can be achieved without the use of a transactivator containing a DNA binding, transcriptional activator domain. A cassette could be assembled to contain a heterologous constitutive promoter downstream of, for example, the URA3 selectable marker, which is flanked with a direct repeat containing homologous sequences to the 5' portion of the target gene. Additional homologous sequences upstream of the target, when added to this cassette would facilitate homologous recombination and replacement of the native promoter with e above-described heterologous promoter cassette immediately upstream of the start codon of the target gene or open reading frame. Conditional expression is achieved by selecting strains, by using 5-FOA containing media, which have excised the heterologous constitutive promoter and
URA3 marker (and consequently lack those regulatory sequences upstream of the target gene required for expression of the gene) and examining the growth of the resulting strain versus a wild type strain grown under identical conditions.

EXAMPLE 14
Promoter Replacement to Generate Cells Capable of Overexpressin or Underexpressing a Gene
Encoding a Gene Product Required for Proliferation
A target for promoter replacement is selected. A promoter replacement cassette is obtained by inserting a nucleic acid comprising the rrnBTIT2 transcriptional terminator followed by the lac promoter into pACYC 184 such that the rrnB terminator and lac promoter are positioned 3'of the
CAT gene. The promoter replacement cassette (CAT-rrnBTIT2-plac) is amplified by PCR. The
PCR product is used as the template for another round of PCR using primers with 60-80 bp of homology to a target promoter (i. e. a promoter which directs expression of a gene encoding a gene product required for proliferation) and 20 bp of homology to the CAT/rrnBTIT2/plac template as described above. The region of homology is chosen such that upon homologous recombination, the CAT/rrnBTIT2/plac cassette replaces the promoter of the target gene but leaves its Shine-Delgarno motif untouched.

The promoter replacement cassette is transformed into competent JC8679. JC8679 is available from the E. coli genetics stock center. JC8679 allows recombination of short linear DNAs and also contains a lacY mutation which allows titratable regulation of the lac promoter. The transformed cells are plated onto LB/chloramphenicol plates containing various levels of IPTG to assure that the correct level of expression is achieved to allow survival. The correct integration of the promoter replacement cassette is confirmed by colony PCR. If desired, proper regulation of the target gene by the inserted promoter may be confirmed by testing the integrants for growth defects when inducer is absent or present at levels lower than that at which the original colonies were obtained. The inability to grow in the absence of inducer (IPTG) or in the presence of lower levels of the inducer than were used to obtain the clones confirms that the target gene is properly regulated by the inserted promoter. It will be appreciated that although the lac promoter and the strain
JC8679 are used as examples, the method may be performed using any suitable regulatable promoter and organism or strain to generate cells which are capable of overexpressing or underexpressing a gene encoding a gene product required for proliferation. Examples of promoters that are useful for the regulating the expression of gene products in Gram-positive organisms over a wide dynamic range are described in U. S. Patent Application Serial Number 10/032, 393, filed
December 21,2001.

The following example describes one method for promoter replacement in a prokaryotic cell. It will be appreciated that promoter replacement can be used in a variety of organisms as previously indicated.

EXAMPLE 15

Operator Insertion to Generate Cells Capable of Overexpressing or Underexpressing a Gene Encoding a Gene Product Required for Proliferation

An oligonucleotide comprising a lac operator flanked on each side by 40 nucleotides homologous to the target promoter is designed. The target promoter is the promoter which drives expression of a gene encoding a gene product required for proliferation, such as the yabB yabCftsL ftsI murE genes in an operon. The sequence of the oligonucleotide (SEQ ID NO. 78582) and locations of the regions homologous to the promoter are illustrated in Figure 6. The sequence of the promoter is also shown with the locations of the-10 and-35 regions indicated (SEQ ID NO.

78583). The single stranded oligonucleotide is transformed into a bacterium expressing the X Beta and Gam proteins. The cells in the transformation mixture are diluted and plated on medium containing IPTG. Colonies in which the lac operator has integrated into the target promoter are identified by colony PCR. If desired, proper regulation of the target promoter by the inserted operator is confirmed by growing the identified colonies in medium containing or lacking IPTG.

The colonies proliferate on medium containing IPTG but fail to grow on medium lacking IPTG, thereby confirming that the target promoter is properly regulated by the inserted operator. It will be appreciated that the preceding method may be performed with any target promoter and any operator to generate cells which overexpress or underexpress a gene encoding a gene product required for proliferation.

EXAMPLE 16

Screening Chemical Libraries

A. Protein-Based Assays

Having isolated and expressed bacterial proteins shown to be required for bacterial proliferation, the present invention further contemplates the use of these expressed target proteins in assays to screen libraries of compounds for potential drug candidates. The generation of chemical libraries is well known in the art. For example, combinatorial chemistry can be used to generate a library of compounds to be screened in the assays described herein. A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis by combining a number of chemical"building block"reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining amino acids in every possible combination to yield peptides of a given length. Millions of chemical compounds theoretically can be synthesized through such combinatorial mixings of chemical building blocks. For example, one commentator observed that the systematic, combinatorial mixing of 100 interchangeable chemical building blocks results in the theoretical synthesis of 100 million tetrameric compounds or 10 billion pentameric compounds. (Gallop et al.,"Applications of Combinatorial Technologies to Drug Discovery, Background and Peptide Combinatorial Libraries,"Journal of Medicinal Chemistry, Vol.

37, No. 9,1233-1250 (1994). Other chemical libraries known to those in the art may also be used, including natural product libraries.

Once generated, combinatorial libraries can be screened for compounds that possess desirable biological properties. For example, compounds which may be useful as drugs or to develop drugs would likely have the ability to bind to the target protein identified, expressed and purified as discussed above. Further, if the identified target protein is an enzyme, candidate compounds would likely interfere with the enzymatic properties of the target protein. For example, the enzymatic function of a target protein may be to serve as a protease, nuclease, phosphatase, dehydrogenase, transporter protein, transcriptional enzyme, and any other type of enzyme known or unknown. Thus, the present invention contemplates using the protein products described above to screen combinatorial chemical libraries.

In one example, the target protein is a serine protease and the substrate of the enzyme is known. The present example is directed towards the analysis of libraries of compounds to identify compounds that function as inhibitors of the target enzyme. First, a library of small molecules is generated using methods of combinatorial library formation well known in the art. U. S. Patent Nos.

5,463,564 and 5,574,656, to Agrafiotis, et al., entitled"System and Method of Automatically Generating Chemical Compounds with Desired Properties,"are two such teachings. Then the library compounds are screened to identify those compounds that possess desired structural and functional properties. U. S. Patent No. 5,684,711, also discusses a method for screening libraries.

To illustrate the screening process, the target polypeptide and chemical compounds of the library are combined with one another and permitted to interact with one another. A labeled substrate is added to the incubation. The label on the substrate is such that a detectable signal is emitted from the products of the substrate molecules that result from the activity of the target polypeptide. The emission of this signal permits one to measure the effect of the combinatorial library compounds on the enzymatic activity of target enzymes by comparing it to the signal emitted in the absence of combinatorial library compounds. The characteristics of each library compound are encoded so that compounds demonstrating activity against the enzyme can be analyzed and features common to the various compounds identified can be isolated and combined into future iterations of libraries.

Once a library of compounds is screened, subsequent libraries are generated using those chemical building blocks that possess the features shown in the first round of screen to have activity against the target enzyme. Using this method, subsequent iterations of candidate compounds will possess more and more of those structural and functional features required to inhibit the function of the target enzyme, until a group of enzyme inhibitors with high specificity for the enzyme can be found.

These compounds can then be further tested for their safety and efficacy as antibiotics for use in mammals.

It will be readily appreciated that this particular screening methodology is exemplary only.

Other methods are well known to those skilled in the art. For example, a wide variety of screening techniques are known for a large number of naturally-occurring targets when the biochemical function of the target protein is known. For example, some techniques involve the generation and use of small peptides to probe and analyze target proteins both biochemically and genetically in order to identify and develop drug leads. Such techniques include the methods described in PCT publications
No. W09935494, W09819162, W09954728. Other techniques utilize natural product libraries or libraries of larger molecules such as proteins.

It will be appreciated that the above protein-based assays may be performed with any of the proliferation-required polypeptides from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhirnurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faeciurn, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the polypeptides of SEQ ID NOs.: 42398-78581) or portions thereof. In addition, the above protein-based assays may be performed with homologous polypeptides or portions thereof.

B. Cell-Based Assays

Current cell-based assays used to identify or to characterize compounds for drug discovery and development frequently depend on detecting the ability of a test compound to modulate the activity of a target molecule located within a cell or located on the surface of a cell. An advantage of cell-based assays is that they allow the effect of a compound on a target molecule's activity to be detected within the physiologically relevant environment of the cell as opposed to an

in vitro environment. Most often such target molecules are proteins such as enzymes, receptors and the like.

However, target molecules may also include other molecules such as DNAs, lipids, carbohydrates and RNAs including messenger RNAs, ribosomal RNAs, tRNAs, regulatory RNAs and the like. A number of highly sensitive cell-based assay methods are available to those of skill in the art to detect binding and interaction of test compounds with specific target molecules. However, these methods are generally not highly effective when the test compound binds to or otherwise interacts with its target molecule with moderate or low affinity. In addition, the target molecule may not be readily accessible to a test compound in solution, such as when the target molecule is located inside the cell or within a cellular compartment. Thus, current cell-based assay methods are limited in that they are not effective in identifying or characterizing compounds that interact with their targets with moderate to low affinity or compounds that interact with targets that are not readily accessible.

The cell-based assay methods of the present invention have substantial advantages over current cell-based assays. These advantages derive from the use of sensitized cells in which the level or activity of at least one proliferation-required gene product (the target molecule) has been specifically reduced to the point where the presence or absence of its function becomes a ratedetermining step for cellular proliferation. Bacterial, fungal, plant, or animal cells can all be used with the present method. Such sensitized cells become much more sensitive to compounds that are active against the affected target molecule. Thus, cell-based assays of the present invention are capable of detecting compounds exhibiting low or moderate potency against the target molecule of interest because such compounds are substantially more potent on sensitized cells than on nonsensitized cells. The effect may be such that a test compound may be two to several times more potent, at least 10 times more potent, at least 20 times more potent, at least 50 times more potent, at least 100 times more potent, at least 1000 times more potent, or even more than 1000 times more potent when tested on the sensitized cells as compared to the non-sensitized cells. The proliferation-required nucleic acids or polypeptides from Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium,
Acinetobacter baumannii, Bacillus antlYacis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Stapltylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasnaa urealyticum, Yibrio cholerae or Yersinia pestis, or portions thereof, may be employed in any of the cell-based assays described herein. Similarly, homologous coding nucleic acids, homologous antisense nucleic acids, or homologous polypeptides or portions of the homologous nucleic acids or homologous polypeptides, may be employed in any of the cell-based assays described herein.

Due in part to the increased appearance of antibiotic resistance in pathogenic microorganisms and to the significant side-effects associated with some currently used antibiotics, novel antibiotics acting at new targets are highly sought after in the art. Yet, another limitation in the current art related to cell-based assays is the problem of repeatedly identifying hits against the same kinds of target molecules in the same limited set of biological pathways. This may occur when compounds acting at such new targets are discarded, ignored or fail to be detected because compounds acting at the"old"targets are encountered more frequently and are more potent than compounds acting at the new targets. As a result, the majority of antibiotics in use currently interact with a relatively small number of target molecules within an even more limited set of biological pathways.

The use of sensitized cells of the current invention provides a solution to the above problem in two ways. First, desired compounds acting at a target of interest, whether a new target or a previously known but poorly exploited target, can now be detected above the"noise"of compounds acting at the"old"targets due to the specific and substantial increase in potency of such desired compounds when tested on the sensitized cells of the current invention. Second, the methods used to sensitize cells to compounds acting at a target of interest may also sensitize these cells to compounds acting at other target molecules within the same biological pathway. For example, expression of an antisense molecule to a gene encoding a ribosomal protein is expected to sensitize the cell to compounds acting at that ribosomal protein and may also sensitize the cells to compounds acting at any of the ribosomal components (proteins or rRNA) or

even to compounds acting at any target which is part of the protein synthesis pathway. Thus an important advantage of the present invention is the ability to reveal new targets and pathways that were previously not readily accessible to drug discovery methods.

Sensitized cells of the present invention are prepared by reducing the activity or level of a target molecule. The target molecule may be a gene product, such as an RNA or polypeptide produced from the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus, Enteococcus faecalis, Klebsiella pneufnoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Carnpylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium di ; gicile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including a gene product produced from the nucleic acids of SEQ ID NOs.: 6214-42397, such as the polypeptides of SEQ ID NOs.: 4239878581) or from homologous nucleic acids. For example, the target molecule may be one of the polypeptides of SEQ ID NOs. 42398-78581 or a homologous polypeptide. Alternatively, the target may be a gene product such as an RNA or polypeptide which is produced from a sequence within the same operon as the proliferation-required nucleic acids Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium diJ0icile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influefzzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella ntultocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or from homologous nucleic acids. In addition, the target may be an RNA or polypeptide in the same biological pathway as the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diphtheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria rraeningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or from homologous nucleic acids. Such biological pathways include, but are not limited to, enzymatic, biochemical and metabolic pathways as well as pathways involved in the production of cellular structures such as the cell wall.

Current methods employed in the arts of medicinal and combinatorial chemistries are able to make use of structure-activity relationship information derived from testing compounds in various biological assays including direct binding assays and cell-based assays. Occasionally compounds are directly identified in such assays that are sufficiently potent to be developed as drugs. More often, initial hit compounds exhibit moderate or low potency. Once a hit compound is

identified with low or moderate potency, directed libraries of compounds are synthesized and tested in order to identify more potent leads. Generally these directed libraries are combinatorial chemical libraries consisting of compounds with structures related to the hit compound but containing systematic variations including additions, subtractions and substitutions of various structural features. When tested for activity against the target molecule, structural features are identified that either alone or in combination with other features enhance or reduce activity. This information is used to design subsequent directed libraries containing compounds with enhanced activity against the target molecule. After one or several iterations of this process, compounds with substantially increased activity against the target molecule are identified and may be further developed as drugs.

This process is facilitated by use of the sensitized cells of the present invention since compounds acting at the selected targets exhibit increased potency in such cell-based assays, thus; more compounds can now be characterized providing more useful information than would be obtained otherwise.

Thus, it is now possible using cell-based assays of the present invention to identify or characterize compounds that previously would not have been readily identified or characterized including compounds that act at targets that previously were not readily exploited using cell-based assays. The process of evolving potent drug leads from initial hit compounds is also substantially improved by the cell-based assays of the present invention because, for the same number of test compounds, more structure-function relationship information is likely to be revealed.

The method of sensitizing a cell entails selecting a suitable gene or operon. A suitable gene or operon is one whose transcription and/or expression is required for the proliferation of the cell to be sensitized. The next step is to introduce into the cells to be sensitized, an antisense RNA capable of hybridizing to the suitable gene or operon or to the RNA encoded by the suitable gene or operon.

Introduction of the antisense RNA can be in the form of a vector in which antisense RNA is produced under the control of an inducible promoter. The amount of antisense RNA produced is modulated by varying an inducer concentration to which the cell is exposed and thereby varying the activity of the promoter driving transcription of the antisense RNA. Thus, cells are sensitized by exposing them to an inducer concentration that results in a sub-lethal level of antisense RNA expression. The requisite amount of inducer may be derived empiracally by one of skill in the art.

In one embodiment of the cell-based assays, antisense nucleic acids complementary to the identified Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salrnonella typhimurium, Acinetobacter baumannii, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Clalamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile,
Corynebacterium diptlaeriae, Efaterobacter cloacae, Efaterococcus faeciurn, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis nucleotide sequences or portions thereof (including antisense nucleic acids comprising a nucleotide sequence complementary to one of SEQ ID NOs.: 6214-42397, and the antisense nucleic acids of SEQ ID
NOs.: 1-6213 or antisense nucleic acids comprising a nucleotide sequence complementary to portions of the foregoing nucleic acids thereof), antisense nucleic complementary to homologous coding nucleic acids or portions thereof or homologous antisense nucleic acids are used to inhibit the production of a proliferation-required protein. Vectors producing antisense RNA complementary to identified genes required for proliferation, or portions thereof, are used to limit the concentration of a proliferation-required protein without severely inhibiting growth. The proliferation-required protein may be one of the proteins of SEQ BD NOs.: 42398-78581 or a homologous polypeptide. To achieve that goal, a growth inhibition dose curve of inducer is calculated by plotting various doses of inducer against the corresponding growth inhibition caused by the antisense expression. From this curve, the concentration of inducer needed to achieve various percentages of antisense induced growth inhibition, from 1 to 100% can be determined.

In some embodiments of the present invention, promoter replacement methods, such as those describe above and in U. S. Patent Application Serial Number 09/948,993, are used to express the proliferation-inhibiting nucleic acid. In other embodiments, the methods for the production of stabilized RNA in Gram-negative organisms, as described in U. S. Provisional Patent Application
Serial Number 60/343,512, are used for the production of proliferation-inhibiting transcripts corresponding to the nucleic acid sequences described herein. Briefly, the stabilized antisense RNA may comprise an antisense RNA which was identified as inhibiting proliferation as described above which has been engineered to contain at least one stem loop flanking each end of the antisense nucleic acid. In some embodiments, the at least one stem-loop structure formed at the 5'end of the stabilized antisense nucleic acid comprises a flush, double stranded 5'end. In some embodiments, one or more of the stem loops comprises a rho independent terminator. In additional embodiments, the stabilized antisense RNA lacks a ribosome binding site. In further embodiments, the stabilized RNA lacks sites which are cleaved by one or more RNAses, such as RNAse E or RNAse III. In some embodiments, the stabilized antisense RNA may be transcribed in a cell which the activity of at least one enzyme involved in RNA degradation has been reduced. For example, the activity of an enzyme such as RNase E, RNase II, RNase III, polynucleotide phosphorylase, and poly (A) polymerase, RNA helicase, enolase or an enzyme having similar functions may be reduced in the cell.

A variety of different regulatable promoters may be used to produce the antisense nucleic acid. Transcription from the regulatable promoters may be modulated by controlling the activity of a transcription factor repressor which acts at the regulatable promoter. For example, if transcription is modulated by affecting the activity of a repressor, the choice of inducer to be used depends on the repressor/operator responsible for regulating transcription of the antisense nucleic acid. If the regulatable promoter comprises a T5 promoter fused to a oxyl0 (xylose operator; e. g. derived from Staphylococcus xylosis (Schnappinger, D. et al., FEMS Microbiol. Let. 129: 126214-423978 (1995), then transcription of the antisense nucleic acid may be regulated by a xylose repressor. The xylose repressor may be provided by ectoptic expression within an S. aureus cell of an exogenous xylose repressor gene, e. g. derived from S. xylosis DNA. In such cases transcription of antisense
RNA from the promoter is inducible by adding xylose to the medium and the promoter is thus "xylose inducible."Similarly, IPTG inducible promoters may be used. For example, the highest concentration of the inducer that does not reduce the growth rate significantly can be estimated from the curve. Cellular proliferation can be monitored by growth medium turbidity via OD measurements. In another example, the concentration of inducer that reduces growth by 25% can be predicted from the curve. In still another example, a concentration of inducer that reduces growth by 50% can be calculated. Additional parameters such as colony forming units (cfu) can be used to measure cellular viability. Some embodiments of the present invention contemplate the use of a vector that comprises a regulatable fusion promoter selected from a suite of fusion promoters wherein the promoter suite is useful for modulating both the basal and maximal levels of transcription of a nucleic acid over a wide dynamic range thus allowing the desired level of production of a transcript which corresponds to a nucleic acid described herein. Such promoters are described in U. S. Patent Application Serial Number 10/032,393, filed December 21,2001, the disclosure of which is incorported herein by reference in its entirety.

Cells to be assayed are exposed to the above-determined concentrations of inducer. The presence of the inducer at this sub-lethal concentration reduces the amount of the proliferation required gene product to a sub-optimal amount in the cell that will still support growth. Cells grown in the presence of this concentration of inducer are therefore specifically more sensitive to inhibitors of the proliferation-required protein or RNA of interest or to inhibitors of proteins or
RNAs in the same biological pathway as the proliferation-required protein or RNA of interest but not to inhibitors of unrelated proteins or RNAs.

Cells pretreated with sub-inhibitory concentrations of inducer and thus containing a reduced amount of proliferation-required target gene product are then used to screen for compounds that reduce cell growth. The sub-lethal concentration of inducer may be any concentration consistent with the intended use of the assay to identify candidate compounds to which the cells are more sensitive. For example, the sub-lethal concentration of the inducer may be such that growth inhibition is at least about 5%, at least about 8%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60% at least about 75%, or more.

Cells which are pre-sensitized using the preceding method are more sensitive to inhibitors of the target protein because these cells contain less target protein

to inhibit than do wild-type cells.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids comprising a nucleotide sequence complementary to any of the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii,
Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes,
Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirahilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typAli, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae,
Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or
Yersinia pestis or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from Escherichia coli,
Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa,
Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis,
Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinurn, Clostridium dcile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae,
Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or homologous polypeptides.

In another embodiment of the cell-based assays of the present invention, the level or activity of a proliferation required gene product is reduced using a mutation, such as a temperature sensitive mutation, in the gene encoding a gene product required for proliferation and an antisense nucleic acid comprising a nucleotide sequence complementary to the gene encoding the gene product required for proliferation or a portion thereof. Growing the cells at an intermediate temperature between the permissive and restrictive temperatures of the temperature sensitive mutant where the mutation is in a proliferation-required gene produces cells with reduced activity of the proliferation-required gene product. The antisense RNA complementary to the proliferationrequired sequence further reduces the activity of the proliferation required gene product. Drugs that may not have been found using either the temperature sensitive mutation or the antisense nucleic acid alone may be identified by determining whether cells in which transcription of the antisense nucleic acid has been induced and which are grown at a temperature between the permissive temperature and the restrictive temperature are substantially more sensitive to a test compound than cells in which expression of the antisense nucleic acid has not been induced and which are grown at a permissive temperature. Also drugs found previously from either the antisense nucleic acid alone or the temperature sensitive mutation alone may have a different sensitivity profile when used in cells combining the two approaches, and that sensitivity profile may indicate a more specific action of the drug in inhibiting one or more activities of the gene product.

Temperature sensitive mutations may be located at different sites within the gene and correspond to different domains of the protein. For example, the dnaB gene of Escherichia coli encodes the replication fork DNA helicase. DnaB has several domains, including domains for oligomerization, ATP hydrolysis, DNA binding, interaction with primase, interaction with DnaC, and interaction with DnaA [(Biswas, E. E. and Biswas, S. B. 1999. Mechanism and DnaB helicase of
Escherichia coli : structural domains involved in ATP hydrolysis, DNA binding, and oligomerization. Biochem. 38: 10919-10928; Hiasa, H. and Marians, K.

J. 1999. Initiation of bidirectional replication at the chromosomal origin is directed by the interaction between helicase and primase. J. Biol. Chem. 274: 27244-27248; San Martin, C., Radermacher, M., Wolpensinger,
B., Engel, A., Miles, C. S., Dixon, N. E., and Carazo, J. M. 1998. Three-dimensional reconstructions from cryoelectron microscopy images reveal an intimate complex between helicase DnaB and its loading partner DnaC. Structure 6: 501-9; Sutton, M. D., Carr, K. M., Vicente, M., and Kaguni, J. M.

1998. Escherichia coli DnaA protein. The N-terminal domain and loading of DnaB helicase at the
E. coli chromosomal origin. J. Biol. Chem. 273: 34255-62.). Temperature sensitive mutations in different domains of DnaB confer different phenotypes at the restrictive temperature, which include either an abrupt stop or slow stop in DNA replication with or without DNA breakdown (Wechsler,
J. A. and Gross, J. D. 1971. Escherichia coli mutants temperature-sensitive for DNA synthesis. Mol.

Gen. Genetics 113: 273-284, and termination of growth or cell death. Combining the use of temperature sensitive mutations in the dnaB gene that cause cell death at the restrictive temperature with an antisense to the dnaB gene could lead to the discovery of very specific and effective inhibitors of one or a subset of activities exhibited by DnaB.

It will be appreciated that the above method may be performed with any mutation which reduces but does not eliminate the activity or level of the gene product which is required for proliferation.

It will be appreciated that the above cell-based assays may be performed using mutations in, such as temperature sensitive mutations, and antisense nucleic acids comprising a nucleotide sequence complementary to any of the genes encoding proliferation-required gene products from from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimuriurn, Acinetobacter baumannii, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei Campylobacter jejuni Chlamydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinurn, Clostridium dicile,
Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi Salmonella typhi Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or portions thereof (including the nucleic acids of SEQ ID NOs.: 6214-42397), mutations in and antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from Escherichia coli Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi Salmonella typhi
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the polypeptides of SEQ ID NOs.: 42398-78581), or homologous polypeptides may be reduced.

When screening for antimicrobial agents against a gene product required for proliferation, growth inhibition of cells containing a limiting amount of that proliferation-required gene product can be assayed. Growth inhibition can be measured by directly comparing the amount of growth, measured by the optical density of the growth medium, between an experimental sample and a control sample. Alternative methods for assaying cell proliferation include measuring the signal from a reporter construct, various enzymatic activity assays, and other methods well known in the art.

It will be appreciated that the above method may be performed in solid phase, liquid phase or a combination of the two. For example, cells grown on nutrient agar containing the inducer of the antisense construct may be exposed to compounds spotted onto the agar surface. If desired, the cells may be grown on agar containing varying concentrations of the inducer. A compound's effect may be judged from the diameter of the resulting killing zone, the area around the compound application point in which cells do not grow. Multiple compounds may be transferred to agar plates and simultaneously tested using automated and semi-automated equipment including but not restricted to multi-channel pipettes (for example the Beckman Multimek) and multi-channel spotters (for example the Genomic Solutions Flexys). In this way multiple plates and thousands to millions of compounds may be tested per day.

The compounds may also be tested entirely in liquid phase using microtiter plates as described below. Liquid phase screening may be performed in microtiter plates containing 96,384, 1536 or more wells per microtiter plate to screen multiple plates and thousands to millions of compounds per day. Automated and semi-automated equipment may be used for addition of reagents (for example cells and compounds) and determination of cell density.

EXAMPLE 17

Cell-Based Assay Using Antisense Complementary to Genes Encoding Ribosomal Proteins

The effectiveness of the above cell-based assay was validated using constructs transribing antisense RNA to the proliferation required E. coli genes rplL, rplJ, and rplW encoding ribosomal proteins L7/L12, L10 and L23 respectively. These proteins are essential components of the protein synthesis apparatus of the cell and as such are required for proliferation. These constructs were used to test the effect of antisense transcription on cell sensitivity to antibiotics known to bind to the ribosome and thereby inhibit protein synthesis. Constructs transcribing antisense RNA to several other genes (elaD, visC, yohH, and atpE/B), the products of which are not involved in protein synthesis were used for comparison.

First, pLex5BA (Krause et al., J. Mol. Biol. 274: 365 (1997), vectors containing antisense constructs to either rplW or to elaD were introduced into separate E. coli cell populations. Vector introduction is a technique well known to those of ordinary skill in the art. The vectors of this example contain IPTG inducible promoters that drive the transcription of the antisense RNA in the presence of the inducer. However, those skilled in the art will appreciate that other inducible promoters may also be used. Suitable vectors are also well known in the art. For example, a number of promoters useful for nucleic acid transcription (including the nucleic acids described herein) in Enterococcus faecalis, Staphylococcus areus as well as other Gram positive organisms are described in U. S. Patent Application Serial Number 10/032,393, filed December 21,2001.

Antisense clones to genes encoding different ribosomal proteins or to genes encoding proteins that are not involved in protein synthesis were utilized to test the effect of antisense transcription on cell sensitivity to the antibiotics known to bind to ribosomal proteins and inhibit protein synthesis.

Antisense nucleic acids comprising a nucleotide sequence complementarty to the elaD, atpB & atpE, visC and John genets are referred to as AS-elaD, AS-atpBlE, AS-visC, AS-yohH respectively.

These genes are not known to be involved in protein synthesis. Antisense nucleic acids to the rplL, rplL & rplJ and rpIW genes are referred to as AS-rplL, AS-rplL/J, and AS-rplW respectively. These genes encode ribosomal proteins L7/L12 (rplL) L10 (repli) and L23 (rplYT). Vectors containing these antisense nucleic acids were introduced into separate E. coli cell populations.

The cell populations containing vectors producing AS-elaD or AS-rplW were exposed to a range of IPTG concentrations in liquid medium to obtain the growth inhibitory dose curve for each clone (Figure 7). First, seed cultures were grown to a particular turbidity measured by the optical density (OD) of the growth solution. The OD of the solution is directly related to the number of bacterial cells contained therein. Subsequently, sixteen 200 ul liquid medium

cultures were grown in a 96 well microtiter plate at 37 C with a range of IPTG concentrations in duplicate two-fold serial dilutions from 1600 uM to 12.5 3 : (final concentration). Additionally, control cells were grown in duplicate without IPTG. These cultures were started from an inoculum of equal amounts of cells derived from the same initial seed culture of a clone of interest. The cells were grown for up to 15 hours and the extent of growth was determined by measuring the optical density of the cultures at 600 nm. When the control culture reached mid-log phase the percent growth (relative to the control culture) for each of the IPTG containing cultures was plotted against the log concentrations of IPTG to produce a growth inhibitory dose response curve for the IPTG. The concentration of IPTG that inhibits cell growth to 50% (IC ; o) as compared to the 0 mM IPTG control (0% growth inhibition) was then calculated from the curve. Under these conditions, an amount of antisense RNA was produced that reduced the expression levels of rplW or elaD to a degree such that growth of cells containing their respective antisense vectors was inhibited by 50%.

Alternative methods of measuring growth are also contemplated. Examples of these methods include measurements of proteins, the expression of which is engineered into the cells being tested and can readily be measured. Examples of such proteins include luciferase and various enzymes.

Cells were pretreated with the selected concentration of IPTG and then used to test the sensitivity of cell populations to tetracycline, erythromycin and other known protein synthesis inhibitors. Figure 7 is an IPTG dose response curve in E. coli transformed with an IPTG-inducible plasmid containing either an antisense clone to the E. coli rplW gene (AS-rplW) which encodes ribosomal protein L23 which is required for protein synthesis and essential for cell proliferation, or an antisense clone to the elaD (AS-elaD) gene which is not known to be involved in protein synthesis.

An example of a tetracycline dose response curve is shown in Figures 8A and 13B for the rplW and elaD genes, respectively. Cells were grown to log phase and then diluted into medium alone or medium containing IPTG at concentrations which give 20% and 50% growth inhibition as determined by IPTG dose response curves. After 2.5 hours, the cells were diluted to a final OD60o of 0.002 into 96 well plates containing (1) +/-IPTG at the same concentrations used for the 2.5 hour pre-incubation; and (2) serial two-fold dilutions of tetracycline such that the final concentrations of tetracycline range from 1 llg/ml to 15.6 ng/ml and 0 llg/rnl. The 96 well plates were incubated at
37 C and the OD6oo was read by a plate reader every 5 minutes for up to 15 hours. For each IPTG concentration and the no IPTG control, tetracycline dose response curves were determined when the control (absence of tetracycline) reached 0.1 OD600.

To compare tetracycline sensitivity with and without IPTG, tetracycline ICsos were determined from the dose response curves (Figures 8A-B). Cells transcribing antisense nucleic acids AS-rplL or AS-rplW to genes encoding ribosomal proteins L7/L12 and L23 respectively showed increased sensitivity to tetracycline (Figure 8A) as compared to cells with reduced levels of the elaD gene product (AS-elaD) (Figure 8B). Figure 9 shows a summary bar chart in which the ratios of tetracycline ICsos determined in the presence of IPTG which gives 50% growth inhibition versus tetracycline ICsos determined without IPTG (fold increase in tetracycline sensitivity) were plotted. Cells with reduced levels of either L7/L12 (encoded by genes rplL, rplJ) or L23 (encoded by the rplW gene) showed increased sensitivity to tetracycline (Figure 9). Cells expressing antisense to genes not known to be involved in protein synthesis (AS-atpBlE, AS-visC, AS-elaD, AS-yohH) did not show the same increased sensitivity to tetracycline, validating the specificity of this assay (Figure 9).

In addition to the above, it has been observed in initial experiments that clones transcribing antisense RNA to genes involved in protein synthesis (including genes encoding ribosomal proteins L7/L12 & L10, L7/L12 alone, L22, and L18, as well as genes encoding rRNA and Elongation
Factor G) have increased sensitivity to the macrolide, erythromycin, whereas clones transcribing antisense to the non-protein synthesis genes elaD, atpBlE and visC do not. Furthermore, the clone transcribing antisense to rplL and rplJ (AS-rplLlJ) does not show increased sensitivity to nalidixic acid and ofloxacin, antibiotics which do not inhibit protein synthesis.

The results with the ribosomal protein genes rplL, rplJ, and role as well as the initial results using various other antisense clones and antibiotics show that limiting the concentration of an antibiotic target makes cells more sensitive to the antimicrobial agents that specifically interact with that protein. The results also show that these cells are sensitized to antimicrobial agents that inhibit the overall function in which the protein target is involved but are not sensitized to antimicrobial agents that inhibit other functions. It will be appreciated that the cell-based assays described above may be implemented using the Escherichia coli, Staphylococcus aureus,

Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhirnurium,
Acinetobacter baurnannii, Bacillus arithracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Clalarnydia pneurnoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium dcile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilsu influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium aviurn, Mycobacteriuna bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis antisense nucleotide sequences which inhibit the activity of genes required for proliferation described herein (including the antisense nucleic acids of SEQ ID NOs.:
1-6213) or antisense nucleic acids comprising nucleotide sequences which are complementary to the sequences of SEQ ID NOs.: 6214-42397 or portions thereof.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorfer, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlafnydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium dicile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphyloccus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii
Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum clostrium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes,
Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized
RNA, as described in U. S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

The cell-based assay described above may also be used to identify the biological pathway in which a proliferation-required nucleic acid or its gene product lies. In such methods, cells transcribing a sub-lethal level of antisense to a target proliferation-required nucleic acid and control cells in which transcription of the antisense has not been induced are contacted with a panel of antibiotics known to act in various pathways. If the antibiotic acts in the pathway in which

the target proliferation-required nucleic acid or its gene product lies, cells in which transcription of the antisense has been induced will be more sensitive to the antibiotic than cells in which expression of the antisense has not been induced.

As a control, the results of the assay may be confirmed by contacting a panel of cells transcribing antisense nucleic acids to many different proliferation-required genes including the target proliferation-required gene. If the antibiotic is acting specifically, heightened sensitivity to the antibiotic will be observed only in the cells transcribing antisense to a target proliferationrequired gene (or cells expressing antisense to other proliferation-required genes in the same pathway as the target proliferation-required gene) but will not be observed generally in all cells expressing antisense to proliferation-required genes.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including antisense nucleic acids complementary to SEQ ID NOs: 6214-42397, or the antisense nucleic acids of SEQ ID NOs.: 1-6213) or portions thereof, antisense nucleic acids comprising nucleotide sequences complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimuriurra, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum,
Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis,
Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the polypeptides of SEQ BD NOs. : 42398-78581), or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized
RNA, as described in U. S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

Similarly, the above method may be used to determine the pathway on which a test compound, such as a test antibiotic acts. A panel of cells, each of which transcribes an antisense to a proliferation-required nucleic acid in a known pathway, is contacted with a compound for which it is desired to determine the pathway on which it acts. The sensitivity of the panel of cells to the test compound is determined in cells in which transcription of the antisense has been induced and in control cells in which expression of the antisense has not been induced. If the test compound acts on the pathway on which an antisense nucleic acid acts, cells in which expression of the antisense has been induced will be more sensitive to the compound than cells in which expression of the antisense has not been induced. In addition, control cells in which expression of antisense to proliferation-required genes in other pathways has been induced will not exhibit heightened sensitivity to the compound. In this way, the pathway on which the test compound acts may be determined.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids comprising nucleotide sequences complementary to any of the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii,
Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium digicile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes,
Moraxella catarrhalis, Mycobacterium aviuna, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae,
Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or
Yersinia pestis (including antisense nucleic acids complementary to SEQ ID NOs : 6214-42397, such as the antisense nucleic acids of SEQ ID NOs. : 1-6213) or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids In this way, the level or activity of a target, such as any of the proliferationrequired polypeptides from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi,
Staphylococcus epidermidis, Staphylococcus haernolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the polypeptides of SEQ ID NOs. : 42398-78581) or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized
RNA, as described in U. S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

The Example below provides one method for performing such assays.

EXAMPLE 18
Identification of the Pathway in which a Proliferation-Required
Gene Lies or the Pathwav on which an Antibiotic Acts
A. Preparation of Bacterial Stocks for Assay
To provide a consistent source of cells to screen, frozen stocks of host bacteria containing the desired antisense construct are prepared using standard microbiological techniques. For example, a single clone of the microorganism can be isolated by streaking out a sample of the original stock onto an agar plate containing nutrients for cell growth and an antibiotic for which the antisense construct contains a selectable marker which confers resistance. After overnight growth an isolated colony is picked from the plate with a sterile needle and transferred to an appropriate liquid growth medium containing the antibiotic required for maintenance of the plasmid. The cells are incubated at 30 C to 37 C with vigorous shaking for 4 to 6 hours to yield a culture in

exponential growth. Sterile glycerol is added to 15% (volume to volume) and lOOuL to 500 L aliquots are distributed into sterile cryotubes, snap frozen in liquid nitrogen, and stored at-80 C for future assays.

B. Growth of Bacteria for Use in the Ass

A day prior to an assay, a stock vial is removed from the freezer, rapidly thawed (37 C water bath) and a loop of culture is streaked out on an agar plate containing nutrients for cell growth and an antibiotic to which the selectable marker of the antisense construct confers resistance. After overnight growth at 37 C, ten randomly chosen, isolated colonies are transferred from the plate (sterile inoculum loop) to a sterile tube containing 5 mL of LB medium containing the antibiotic to which the antisense vector confers resistance. After vigorous mixing to form a homogeneous cell suspension, the optical density of the suspension is measured at 600 nm (OD600) and if necessary an aliquot of the suspension is diluted into a second tube of 5 mL, sterile, LB medium plus antibiotic to achieve an OD600 < 0.02 absorbance units. The culture is then incubated at 37 C for 1-2 hrs with shaking until the OD600 reaches OD 0.2-0.3. At this point the cells are ready to be used in the assay.

C. Selection of Media to be Used in Assav

Two-fold dilution series of the inducer are generated in culture media containing the appropriate antibiotic for maintenance of the antisense construct. Several media are tested side by side and three to four wells are used to evaluate the effects of the inducer at each concentration in each media. For example, LB broth, TBD broth and Muller-Hinton media may be tested with the inducer xylose at the following concentrations, 5 mM, 10 mM, 20 mM, 40 mM, 80 mM, 120 mM and 160 mM. Equal volumes of test media-inducer and cells are added to the wells of a 384 well microtiter plate and mixed. The cells are prepared as described above and diluted 1: 100 in the appropriate media containing the test antibiotic immediately prior to addition to the microtiter plate wells. For a control, cells are also added to several wells of each media that do not contain inducer, for example 0 mM xylose. Cell growth is monitored continuously by incubation at 37 C in a microtiter plate reader monitoring the OD600 of the wells over an 18-hour period. The percent inhibition of growth produced by each concentration of inducer is calculated by comparing the rates of logarithmic growth against that exhibited by cells growing in medium without inducer. The medium yielding greatest sensitivity to inducer is selected for use in the assays described below.

D. Measurement of Test Antibiotic Sensitivity in the Absence of Antisense Construct Induction

Two-fold dilution series of antibiotics of known mechanism of action are generated in the culture medium selected for further assay development that has been supplemented with the antibiotic used to maintain the construct. A panel of test antibiotics known to act on different pathways is tested side by side with three to four wells being used to evaluate the effect of a test antibiotic on cell growth at each concentration. Equal volumes of test antibiotic and cells are added to the wells of a 384 well microtiter plate and mixed. Cells are prepared as described above using the medium selected for assay development supplemented with the antibiotic required to maintain the antisense construct and are diluted 1: 100 in identical medium immediately prior to addition to the microtiter plate wells. For a control, cells are also added to several wells that lack antibiotic, but contain the solvent used to dissolve the antibiotics. Cell growth is monitored continuously by incubation at 37 C in a microtiter plate reader monitoring the OD600 of the wells over an 18-hour period. The percent inhibition of growth produced by each concentration of antibiotic is calculated by comparing the rates of logarithmic growth against that exhibited by cells growing in medium without antibiotic. A plot of percent inhibition against log [antibiotic concentration] allows extrapolation of an ICso value for each antibiotic.

E. Measurement of Test Antibiotic Sensitivity in the Presence of Antisense Construct Inducer

The culture medium selected for use in the assay is supplemented with inducer at concentrations shown to inhibit cell growth by 50% and 80% as described above, as well as the antibiotic used to maintain the construct. Two-fold dilution series of the panel of test antibiotics used above are generated in each of these media. Several antibiotics are tested side by side in each medium with three to four wells being used to evaluate the effects of an antibiotic on cell growth at each concentration. Equal volumes of test antibiotic and cells are added to the wells of a 384 well microtiter plate and mixed. Cells are prepared as described above using the medium selected for use in the assay supplemented with the antibiotic required to maintain the antisense construct. The cells are diluted 1: 100 into two 50 mL aliquots of identical medium containing concentrations of inducer that have been shown to inhibit cell growth by 50% and 80 % respectively and incubated at 37 C with shaking for 2.5 hours. Immediately prior to addition to the microtiter plate wells, the cultures are adjusted to an appropriate OD600 (typically 0.002) by dilution into warm (37 C) sterile medium supplemented with identical concentrations of the inducer and antibiotic

used to maintain the antisense construct. For a control, cells are also added to several wells that contain solvent used to dissolve test antibiotics but which contain no antibiotic. Cell growth is monitored continuously by incubation at 37 C in a microtiter plate reader monitoring the OD600 of the wells over an 18-hour period. The percent inhibition of growth produced by each concentration of antibiotic is calculated by comparing the rates of logarithmic growth against that exhibited by cells growing in medium without antibiotic. A plot of percent inhibition against log [antibiotic concentration] allows extrapolation of an ICSO value for each antibiotic.

F. Determining the Specificitv of the Test Antibiotics

A comparison of the ICsos generated by antibiotics of known mechanism of action under antisense induced and non-induced conditions allows the pathway in which a proliferation-required nucleic acid lies to be identified. If cells expressing an antisense nucleic acid comprising a nucleotide sequence complementary to a proliferation-required gene are selectively sensitive to an antibiotic acting via a particular pathway, then the gene against which the antisense acts is involved in the pathway on which the antibiotic acts.

G. Identification of Pathway in which a Test Antibiotic Acts

As discussed above, the cell-based assay may also be used to determine the pathway against which a test antibiotic acts. In such an analysis, the pathways against which each member of a panel of antisense nucleic acids acts are identified as described above. A panel of cells, each containing an inducible vector which transcribes an antisense nucleic acid comprising a nucleotide sequence complementary to a gene in a known proliferation-required pathway, is contacted with a test antibiotic for which it is desired to determine the pathway on which it acts under inducing and non-inducing conditions. If heightened sensitivity is observed in induced cells transcribing antisense complementary to a gene in a particular pathway but not in induced cells transcribing antisense nucleic acids comprising nucleotide sequences complementary to genes in other pathways, then the test antibiotic acts against the pathway for which heightened sensitivity was observed.

One skilled in the art will appreciate that further optimization of the assay conditions, such as the concentration of inducer used to induce antisense transcription and/or the growth conditions used for the assay (for example incubation temperature and medium components) may further increase the selectivity and/or magnitude of the antibiotic sensitization exhibited.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids comprising nucleotide sequences complementary to any of the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii,
Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium,
Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes,
Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae,
Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or
Yersinia pestis (including antisense nucleic acids comprising nucleotide sequences complemenatary to SEQ ID NOs: 6214-42397, such as the antisense nucleic acids of SEQ ID NOs.: 1-6213) or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, corynebacterium diptheriae,

Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae,
Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi,
Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the polypeptides of SEQ ID NOs.: 42398-78581), or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized
RNA, as described in U. S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

The following example confirms the effectiveness of the methods described above.

EXAMPLE 19
Identification of the Biological Pathway in which a Proliferation-Required Gene Lies
The effectiveness of the above assays was validated using proliferation-required genes from
E. coli which were identified using procedures similar to those described above. Antibiotics of various chemical classes and modes of action were purchased from Sigma Chemicals (St. Louis,
MO). Stock solutions were prepared by dissolving each antibiotic in an appropriate aqueous solution based on information provided by the manufacturer. The final working solution of each antibiotic contained no more than 0.2% (w/v) of any organic solvent. To determine their potency against a bacterial strain engineered for transcription of an antisense comprising a nucleotide sequence complementary to a proliferation-required 50S ribosomal protein, each antibiotic was serially diluted two-or three-fold in growth medium supplemented with the appropriate antibiotic for maintenance of the antisense construct. At least ten dilutions were prepared for each antibiotic.

25 liL aliquots of each dilution were transferred to discrete wells of a 384-well microplate (the assay plate) using a multi-channel pipette. Quadruplicate wells were used for each dilution of an antibiotic under each treatment condition (plus and minus inducer). Each assay plate contained twenty wells for cell growth controls (growth medium replacing antibiotic), ten wells for each treatment (plus and minus inducer, in this example IPTG). Assay plates were usually divided into the two treatments: half the plate containing induced cells and an appropriate concentrations of inducer (in this example IPTG) to maintain the state of induction, the other half containing noninduced cells in the absence of IPTG.

Cells for the assay were prepared as follows. Bacterial cells containing a construct, from which transcription of antisense nucleic acid comprising a nucleotide sequence complementary to rpIL and rplJ (AS-rplLlJ), which encode proliferation-required 50S ribosomal subunit proteins, is inducible in the presence of IPTG, were grown into exponential growth (OD600 0.2 to 0.3) and then diluted 1: 100 into fresh medium containing either 400 uM or 0 uM inducer (IPTG). These cultures were incubated at 37 C for 2.5 hr. After a 2.5 hr incubation, induced and non-induced cells were respectively diluted into an assay medium at a final OD600 value of 0.0004. The medium contained an appropriate concentration of the antibiotic for the maintenance of the antisense construct. In addition, the medium used to dilute induced cells was supplemented with 800 p IPTG so that addition to the assay plate would result in a final IPTG concentration of 400 uM. Induced and noninduced cell suspensions were dispensed (25 IIVwell) into the appropriate wells of the assay plate as discussed previously. The plate was then loaded into a plate reader, incubated at constant temperature, and cell growth was monitored in each well by the measurement of light scattering at 595 nm. Growth was monitored every 5 minutes until the cell culture attained a stationary growth phase. For each concentration of antibiotic, a percentage inhibition of growth was calculated at the time point corresponding to mid-exponential growth for the associated control wells (no antibiotic, plus or minus IPTG). For each antibioti or minus IPTG), a plot of percent inhibition versus log of antibiotic concentration was generated and the ICso determined. A comparison of the ICso for each antibiotic in the presence and absence of IPTG revealed whether induction of the antisense construct sensitized the cell to the mechanism of action exhibited by the antibiotic. Cells which exhibited a statistically significant decrease in the ICso value in the presence of inducer were

considered to have an increased sensitivity to the test antibiotic.

The results are provided in the table below, which lists the classes and names of the antibiotics used in the analysis, the targets of the antibiotics, the ICso in the absence of IPTG, the ICso in the presence of IPTG, the concentration units for the ICsos, the fold increase in ICSO in the presence of IPTG, and whether increased sensitivity was observed in the presence of IPTG.

TABLE V
Effect of Expression of Antisense RNA to rplL and rplJ on Antibiotic Sensitivity
EMI1558.1

ANTIBIOTIC CLASS /Name	TARGET	IC50 (-IPTG)	IC50 (+IPTG)	Conc. Unit	Fold Increase in Sensitivity	Sensitivity Increased?
PROTEIN SYNTHESIS INHIBITOR						
AMINOGLYCOSIDES						
Gentamicin	30S ribosome function	2715	19.19	ng/ml	141	Yes
Streptomycin	30S ribosome function	11280	161	ng/ml	70	Yes
Spectinomycin	30S ribosome function	18050	<156	ng/ml		Yes
tobramycin	30S ribosome function	3594	70.58	ng/ml	51	Yes
MACROLIDES						
Erythromycin	50S ribosome function	7467	187	ng/ml	40	Yes
AROMATIC POYKETIDES						
Tetracycline	30S ribosome function	199.7	1.83	ng/ml	109	Yes
Minocycline	30S ribosome function	668.4	3.897	ng/ml	172	Yes
Doxycycline	30S ribosome function	413.1	27.81	ng/ml	15	Yes
OTHER PROTEIN SYNTHESIS INHIBITORS						
Fusidic acid	Elongation Factor G function	59990	641	ng/ml	94	Yes
Chloramphenicol	30S ribosome function	465.4	1.516	ng/ml	307	Yes

Lincomycin 50S ribosome function 47150 324.2 ng/ml 145 Yes

OTHER ANTIBIOTIC MECHANSMS

B-LACTAMS

Cefoxitin Cell wall biosynthesis 2782 2484 ng/ml 1 No

Cefotaxime Cell wall biosynthesis 2782 2484 ng/ml 1 No

DNA SYNTHESIS INHIBITORS

Nalidixic acid DNA Gyrase activity 6973 6024 ng/ml 1 No

Ofloxacin DNA Gyrase activity 49.61 45.89 ng/ml 1 No

OTHER

Bacitracin Cell membrane function 4077 4677 mg/ml 1 No

Trimethoprim Dihydrofolate Reductase activity 128.9 181,.97 ng/ml 1 No

Vancomycin Cell wall biosynthesis 145400 72500 ng/ml 2 No

The above results demonstrate that induction of an antisense RNA complementary to genes encoding 50S ribosomal subunit proteins results in a selective and highly significant sensitization of cells to antibiotics that inhibit ribosomal function and protein synthesis. The above results further demonstrate that induction of an antisense to an essential gene sensitizes a cell or microorganism to compounds that interfere with that gene product's biological role. This sensitization is restricted to compounds that interfere with pathways associated with the targeted gene and its product.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borreliak burgdorferi, burkholderia cepacia, Burkholderia fungorurn, Burkholderia mallei, Canapylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haetnophilus iizfluenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Myucoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including antisense nucleic acids complementary to SEQ ID NOs. 6214-42397, such as the antisense nucleic acids of SEQ ID
NOs.: 1-6213) or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from

Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhifnurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicuna, Clostridium botulinum,
Clostridium dzcile, Corynebacterium diptlaeriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrl2oeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the polypeptides of SEQ ID NOs.: 42398-78581), or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U. S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

Example 20 below describes an analysis performed in Staphylococcus aureus.

EXAMPLE 20
Identification of the Biological Pathway in which a Gene Required for
Proliferation of Staphvlococcus aureus Lies
Antibiotics of various chemical classes and modes of action were purchased from chemical suppliers, for example Sigma Chemicals (St. Louis, MO). Stock solutions were prepared by dissolving each antibiotic in an appropriate aqueous solution based on information provided by the manufacturer. The final working solution of each antibiotic contained no more than 0.2% (w/v) of any organic solvent.

To determine its potency against a bacterial strain containing an antisense nucleic acid comprising a nucleotide sequence complementary to the nucleotide sequence encoding the Beta subunit of DNA gyrase (which is required for proliferation) under the control of a xylose inducible promoter, each antibiotic was serially diluted two-or three-fold in growth medium supplemented with the appropriate antibiotic for maintenance of the antisense construct. At least ten dilutions were prepared for each antibiotic.

Aliquots (25 uL) of each dilution were transferred to discrete wells of a 384-well microplate (the assay plate) using a multi-channel pipette. Quadruplicate wells were used for each dilution of an antibiotic under each treatment condition (plus and minus inducer). Each assay plate contained twenty wells for cell growth controls (growth medium, no antibiotic), ten wells for each treatment (plus and minus inducer, xylose, in this example). Half the assay plate contained induced cells (in this example Staphylococcus aureus cells) and appropriate concentrations of inducer (xylose, in this example) to maintain the state of induction while the other half of the assay plate contained non-induced cells maintained in the absence of inducer.

Preparation of Bacterial Cells
Cells of a bacterial clone containing a construct in which transcription of antisense comprising a nucleotide sequence complementary to the sequence encoding the Beta subunit of
DNA gyrase under the control of the xylose inducible promoter (S1M10000001F08) were grown into exponential growth (OD6oo 0. 2 to 0.3) and then diluted 1: 100 into fresh medium containing either 12 mM or 0 mM inducer (xylose). These cultures were incubated at 37 C for 2.5 hr. The presence of inducer (xylose) in the medium initiates and maintains production of antisense RNA from the antisense construct. After a 2.5 hr incubation, induced and noninduced cells were respectively diluted into an assay medium containing an appropriate concentration of the antibiotic for the maintenance of the antisense construct. In addition, medium used to dilute induced cells was supplemented with 24 mM xylose so that addition to the assay plate would result in

a final xylose concentration of 12 mM. The cells were diluted to a final OD600 value of 0.0004.

Induced and non-induced cell suspensions were dispensed (25 IIVwell) into the appropriate wells of the assay plate as discussed previously. The plate was then loaded into a plate reader and incubated at constant temperature while cell growth was monitored in each well by the measurement of light scattering at 595 nm. Growth was monitored every 5 minutes until the cell culture attained a stationary growth phase. For each concentration of antibiotic, a percentage inhibition of growth was calculated at the time point corresponding to midexponential growth for the associated control wells (no antibiotic, plus or minus xylose). For each antibiotic and condition (plus or minus xylose), plots of percent inhibition versus Log of antibiotic concentration were generated and ICsos determined.

A comparison of each antibiotic's ICso in the presence and absence of inducer (xylose, in this example) reveals whether induction of the antisense construct sensitized the cell to the antibiotic's mechanism of action. If the antibiotic acts against the (3 subunit of DNA gyrase, the ICso of induced cells will be significantly lower than the ICso of uninduced cells.

Figure 10 lists the antibiotics tested, their targets, and their fold increase in potency between induced cells and uninduced cells. As illustrated in Figure 10, the potency of cefotaxime, cefoxitin, fusidic acid, lincomycin, tobramycin, trimethoprim and vancomycin, each of which act on targets other than the ss subunit of gyrase, was not significantly different in induced cells as compared to uninduced cells. However, the potency of novobiocin, which is known to act against the Beta subunit of DNA gyrase, was significantly different between induced cells and uninduced cells.

Thus, induction of an antisense nucleic acid comprising a nucleotide sequence complementary to the sequence encoding the ss subunit of gyrase results in a selective and significant sensitization of Staphylococcus aureus cells to an antibiotic which inhibits the activity of this protein. Furthermore, the results demonstrate that induction of an antisense construct to an essential gene sensitizes a cell or microorganism to compounds that interfere with that gene product's biological role. This sensitization is apparently restricted to compounds that interfere with the targeted gene and its product.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including antisense nucleic acids complementary to SEQ ID NOs.: 6214-42397, such as the antisense nucleic acids of SEQ ID
NOs. 1-6213), or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides : from
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella cataf laalis, Mycobacterium aviurn, Mycobacterium bovis,

Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U. S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

Assays utilizing antisense constructs to essential genes or portions thereof can be used to identify compounds that interfere with the activity of those gene products. Such assays could be used to identify drug leads, for example antibiotics.

Panels of cells transcribing different antisense nucleic acids can be used to characterize the point of intervention of a compound affecting an essential biochemical pathway including antibiotics with no known mechanism of action.

Assays utilizing antisense constructs to essential genes can be used to identify compounds that specifically interfere with the activity of multiple targets in a pathway. Such constructs can be used to simultaneously screen a sample against multiple targets in one pathway in one reaction (Combinatorial HTS).

Furthermore, as discussed above, panels of antisense construct-containing cells may be used to characterize the point of intervention of any compound affecting an essential biological pathway including antibiotics with no known mechanism of action.

It will be appreciated that the above cell-based assays may be performed using antisense nucleic acids complementary to any of the proliferation-required nucleic acids from
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium dicile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus hlfluestzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitaliu7n, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including antisense nucleic acids comprising nucleotide sequences complementary to SEQ ID NOs.: 6214-42397, such as the antisense nucleic acids of SEQ ID NOs. 1-6213), or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids or portions thereof, or homologous antisense nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from Eschericl1ia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia penumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faeciunt, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrlzoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,

Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or homologous polypeptides may be reduced.

In some embodiments of the present invention, the methods for the production of stabilized RNA, as described in U. S. Provisional Patent Application Serial Number 60/343,512, can be used in the above cell-based assays in Gram-negative organisms to extend the lifetime of transcripts corresponding to the nucleic acids described herein.

Another embodiment of the present invention is a method for determining the pathway against which a test antibiotic compound is active, in which the activity of target proteins or nucleic acids involved in proliferation-required pathways is reduced by contacting cells with a sub-lethal concentration of a known antibiotic which acts against the target protein or nucleic acid. In one embodiment, the target protein or nucleic acid corresponds to a proliferationrequired nucleic acid identified using the methods described above, such as the polypeptides of
SEQ ID NOs.: 42398-78581, or homologous polypeptides. The method is similar to those described above for determining which pathway a test antibiotic acts against, except that rather than reducing the activity or level of a proliferation-required gene product using a sub-lethal level of antisense to a proliferation-required nucleic acid, the sensitized cell is generated by reducing the activity or level of the proliferation-required gene product using a sub-lethal level of a known antibiotic which acts against the proliferation required gene product. Heightened sensitivity determines the pathway on which the test compound is active.

Interactions between drugs which affect the same biological pathway have been described in the literature. For example, Mecillinam (Amdinocillin) binds to and inactivates the penicillin binding protein 2 (PBP2, product of the mrdS in E. coli). This antibiotic interacts with other antibiotics that inhibit PBP2 as well as antibiotics that inhibit other penicillin binding proteins such as PBP3 [(Gutmann, L., Vincent, S., Billot-Klein, D., Acar, J. F., Mrena, E., and Williamson, R. (1986) Involvement of penicillin-binding protein 2 with other penicillin-binding proteins in lysis of Escherichia coli by some beta-lactam antibiotics alone and in synergistic lytic effect of amdinocillin (mecillinam). Antimicrobial Agents & Chemotherapy, 30: 906-912).

Interactions between drugs could, therefore, involve two drugs that inhibit the same target protein or nucleic acid or inhibit different proteins or nucleic acids in the same pathway [(Fukuoka, T., Domon, H., Kakuta, M., Ishii, C., Hirasawa, A., Utsui, Y., Ohya, S., and Yasuda,
H. (1997) Combination effect between panipenem and vancomycin on highly methicillinresistant Staphylococcus aureus. Japan. J. Antibio. 50: 411-419; Smith, C. E., Foleno, B. E.,
Barrett, J. F., and Frosc, M. B. (1997) Assessment of the synergistic interactions of levofloxacin and ampicillin against Enterococcus faecium by the checkerboard agar dilution and time-kill methods. Diagnos. Microbiol. Infect. Disease 27: 85-92; den Hollander, J. G., Horrevorts, A. M., van Goor, M. L., Verbrugh, H. A., and Mouton, J. W. (1997) Synergism between tobramycin and ceftazidime against a resistant Pseudomonas aeruginosa strain, tested in an in vitro pharmacokinetic model. Antimicrobial Agents & Chemotherapy. 41: 95-110).

Two drugs may interact even though they inhibit different targets. For example, the proton pump inhibitor, Omeprazole, and the antibiotic, Amoxycillin, two synergistic compounds acting together, can cure Helicobacter pylori infection [(Gabryelewicz, A.,
Laszewicz, W., Dzieniszewski, J., Ciok, J., Marlicz, K., Bielecki, D., Popiela, T., Legutko, J., Knapik, Z., Poniewierka, E. (1997) Multicenter evaluation of dual-therapy (omeprazol and amoxycillin) for Helicobacter pylori-associated duodenal and gastric ulcer (two years of the observation). J. Physiol. Pharmacol. 48 Suppl 4: 93-105).

The growth inhibition from the sub-lethal concentration of the known antibiotic may be at least about 5%, at least about 8%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, or at least about 75%, or more.

Alternatively, the sub-lethal concentration of the known antibiotic may be determined by measuring the activity of the target proliferation-required gene product rather than by measuring growth inhibition.

* Cells are contacted with a combination of each member of a panel of known antibiotics at a sub-lethal level and varying concentrations of the test antibiotic. As a control, the cells are contacted with varying concentrations of the test antibiotic alone. The IC50 of the test antibiotic in the presence and absence of the known antibiotic is determined. If the ICsos in the presence and absence of the known drug are substantially similar, then the test drug and the known drug act on different pathways. If the IC50s are substantially different, then the test drug and the known drug act on the same pathway.

It will be appreciated that the above cell-based assays may be performed using a sublethal concentration of a known antibiotic which acts against the product of any of the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baurnannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium dicile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the products of SEQ ID NOs: 6214-42397, or portions thereof, or the products of homologous coding nucleic acids or portions thereof. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faeciurn, Haemophilus ifzfluenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella pararyphi, Salmonella tphi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis (including the polypeptides of SEQ II) NOs.: 42398-78581), or homologous polypeptides may be reduced.

Another embodiment of the present invention is a method for identifying a candidate compound for use as an antibiotic in which the activity of target proteins or nucleic acids involved in proliferation-required pathways is reduced by contacting cells with a sub-lethal concentration of a known antibiotic which acts against the target protein or nucleic acid. In one embodiment, the target protein or nucleic acid is a target protein or nucleic acid corresponding to a proliferation-required nucleic acid identified using the methods described above. The method is similar to those described previously herein for identifying candidate compounds for use as antibiotics except that rather than reducing the activity or level of a proliferation-required gene product using a sub-lethal level of antisense to a proliferation-required nucleic acid, the activity or level of the proliferation-required gene product is reduced using a sub-lethal level of a known antibiotic which acts against the proliferation required gene product.

The growth inhibition from the sub-lethal concentration of the known antibiotic may be at least about 5%, at least about 8%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, or at least about 75%, or more.

Alternatively, the sub-lethal concentration of the known antibiotic may be determined by measuring the activity of the target proliferation-required gene product rather than by measuring growth inhibition.

In order to characterize test compounds of interest, cells are contacted with a panel of known antibiotics at a sub-lethal level and one or more concentrations of the test compound. As a control, the cells are contacted with the same concentrations of the test compound alone. The ICso of the test compound in the presence and absence of the known antibiotic is determined. If the IC50 of the test compound is substantially different in the presence and absence of the known drug then the test compound is a good candidate for use as an antibiotic. As discussed above, once a candidate compound is identified using the above methods its structure may be optimized using standard techniques such as combinatorial chemistry.

Representative known antibiotics which may be used in each of the above methods are provided in Table VI below. However, it will be appreciated that other antibiotics may also be used.

TABLE VI
Antibiotics and Their Targets ANTIBIOTICINHIBITS/TARGETRESISTANT
MUTANTS
Inhibitors of Transcription
Rifamycin, Rifampicin Inhibits initiation of transcription/3-rpoB, crp, cyaA
Rifabutin Rifaximin subunit RNA polymerase, rpoB
Streptolydigin Accelerates transcription chain rpoB termination/B-subunit RNA polymerase
Streptovaricin an acyclic ansamycin, inhibits RNA rpoB polymerase
Actinomycin D+EDTA Intercalates between 2 successive G-C pldA pairs, rpoB, inhibits RNA synthesis
Inhibitors of Nucleic Acid Metabolism
Quinolones, a subunit gyrase and/or topoisomerase
Nalidixic acid Oxolinic IV, gyrA gyrAorB, icd, sloB acid
Fluoroquinolones a subunit gyrase, gyrA and/or gyrA
Ciprofloxacin, topoisomerase IV (probable target in norA (efflux in
Norfloxacin Staph) Staph) hipQ
Coumerins Inhibits ATPase activity of B-subunit
Novobiocin gyrase, gyrB gyrB, cysB, cysE, nov, 07npA
Coumermycin Inhibits ATPase activity of B-subunit gyrB, hisW gyrase, gyrB
Albicidin DNA synthesis tsx (nucleoside channel)
Metronidazole Causes single-strand breaks in DNA nar
Inhibitors of Metabolic Pathways
Sulfonamides, blocks synthesis of folP, gpt, pabA, Sulfanilamide dihydrofolate, dihydro-pteroate pabB, pabC synthesis,folk
Trimethoprim, Inhibits dihydrofolate reductase, folaA folA, thyA Showdomycin Nucleoside analogue capable of nupC, pnp alkylating sulfhydryl groups, inhibitor of thymidylate synthetase
Thiolactomycin type II fatty acid synthase inhibitor emrB fadB, emrB due to gene dosage
Psicofuranine Adenosine glycoside antibiotic, target is guaA, B
GMP synthetase
Triclosan Inhibits fatty acid synthesis fabi (envM)
Diazoborines Isoniazid, heterocyclic, contain boron, inhibit fatty fabl (envM)
Ethionamide acid synthesis, enoyl-ACP reductase, fabl
Inhibitors of Translation
Phenylpropanoids Binds to ribosomal peptidyl transfer
Chloramphenicol, center preventing peptide translocation/rrn, cmlA, marA, binds to S6, L3, L6, L14, L16, L25, ompF, ompR
L26, L27, but preferentially to L16

Tetracyclines, type II Binding to 30S ribosomal subunit,"A"sil clmA (cmr), mar, polyketides on 30S subunit, blocks peptide ompF Minocycline elongation, strongest binding to S7
Doxycycline
Macrolides (type I Binding to 50 S ribosomal subunit, 23S polyketides) rRNA, blocks peptide translocation,
Erythromycin, L15, L4, L12 rrn, rplC, rplD, rplV,
Carbomycin, mac
Spiramycin etc
Aminoglycosides Irreversible binding to 30S ribosomal
Streptomycin, subunit, prevents translation or causes rpsL, strC, M, ubiF mistranslation of mRNA/16S rRNA atpA-E, ecfB,
Neomycin 12emAC, D, E, G, topA,
Spectincomycin rpsC, D, E, rrn, spcB atpA-atpE, cpxA,
Kanamycin ecfB, hemA,B,L, topA ksgA, B, C, D, rplB, K,
Kasugamycin rpsI,N,M,R rplF, ubiF
Gentamicin,
Amikacin cpxA
Paromycin rpsL
Lincosamides Binding to 50 S ribosomal subunit,
Lincomycin, blocks peptide translocation linB, rplN, 0, rpsG
Clindamycin
Streptogramins 2 components, Streptogramins A & B,
Virginiamycin, bind to the 50S ribosomal subunit
Pristinamycin blocking peptide translocation and
Synercid: quinupristin peptide bond formation /dalfopristin
Fusidanes Inhibition of elongation factor G (EF-G) fusA
Fusidic Acid prevents peptide translocation
Kirromycin (Mocimycin) Inhibition of elongation factor TU (EF-tufA, B
Tu), prevents peptide bond formation
Pulvomycin Binds to and inhibits EF-TU
Thiopeptin Sulfur-containing antibiotic, inhibits rplE protein synthesis, EF-G
Tiamulin Inhibits protein synthesis rplC, rplD Negamycin Inhibits termination process of protein prfB synthesis
Oxazolidinones Linezolid 23S rRNA
Isoniazid pdx
Nitrofurantoin Inhibits protein synthesis, nfA,B nitroreductases convert nitrofurantoin to highly reactive electrophilic intermediates which attack bacterial ribosomal proteins non-specifically
Pseudomonic Acids Inhibition of isoleucyl tRNA ileS
Mupirocin (Bactroban) synthetase-used for Staph, topical cream, nasal spray
Indolmycin Inhibits tryptophanyl-tRNA synthetase trpS
Viomycin rrrnA (23S rRNA methyltransferase ; mutant has slow growth rate, slow chain elongation rate, and viomycin resistance)
Thiopeptides Binds to L11-23S RNA complex
Thiostrepton Inhibits GTP hydrolysis by EF-G
Stimulates GTP hydrolysis by EF-G
Micrococcin
Inhibitors of Cell Walls/Membranes 13-lactams Inhibition of one or more cell wall

Penicillin, Ampicillin transpeptidases, endopeptidases, and
Methicillin, glycosidases (PBPs), of the 12 PBPs ampC, ampD, ample, only 2 are essential : mrdA (PBP2) and envZ, galU, hipA, ftsl (pbpB, PBP3) hipQ, ompC, ompF, ompR, past, rfa, tolD, tolE
Cephalosporins, tonB
Binds to and inactivates PBP2 (mrdA) alaS, argS, crp, cyaA,
Mecillinam (amdinocillin)
Inactivates PBP3 (ftsI) envB, mrdA,B, mreB,C,D
Aztreonam (Furazlocillin) mreB,C,D
Bacilysin, Tetaine Dipeptide, inhib glucosamine dppA synthase
Glycopeptides Vancomycin, Inhib G+ cell wall syn, binds to terminal D-ala-D-ala of pentapeptide,
Polypeptides Bacitracin Prevents dephosphorylation and regeneration of lipid carrier rfa
Cyclic lipopeptide Disrupts multiple aspects of
Daptomycin, membrane function, including peptidoglycan synthesis, lipoteichoic acid synthesis, and the bacterial membrane potential
Cyclic polypeptides Surfactant action disrupts cell pmrA
Polymixin, membrane lipids, binds lipid A mioety of LPS Fosfomycin, Analogue of P-enolpyruvate, inhibits murA, crp, cyaA 15 step in peptidoglycan synthesis-glpT, hipA, ptsl,
UDP-N-acetylglucosamine uhpT enolpyruvyl transferase, murA. Also acts as Immunosuppressant
Cycloserine Prevents formation of D-ala dimer, hipA, cycA inhibits D-ala ligase, ddlA, B
Alafosfalin phosphonodipeptide, cellwall pepA, tpp synthesis inhibitor, potentiator of ss- lactams
Inhibitors of Protein Processing/Transport
Globomycin Inhibits signal peptidase II (cleaves lpp, dnaE prolipoproteins subsequent to lipid modification, IspA
It will be appreciated that the above cell-based assays may be performed using a sublethal concentration of a known antibiotic which acts against the product of any of the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium aviutn, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or portions thereof, or homologous nucleic acids. In this way, the level or activity of a target, such as any of the proliferation-required polypeptides from Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter bauynannii, Bacillus ant7xracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia rnallei, Campylobacter jejuni, Chlanzydia pneumoniae, Chlarnydia trachornatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium dcile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitaliurn, Mycoplasma pneumoniae, Neisseria gonorrltoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,

Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or homologous polypeptides may be reduced.

EXAMPLE 21

Strains in which a Gene Encoding a Gene Product Required for Proliferation is Overexpressed are able to Grow at Elevated Antibiotic Concentrations

To confirm that cells which overexpress a gene product required for proliferation are able to grow at elevated antibiotic concentrations, 11 such genes from Staphylococcus aureus which are the targets of known antibiotics were operably linked to the xylose inducible promoter XylT5 (described in U. S. Patent Application Serial Number 10/032,393. The genes and the antibiotics which target the products of these genes are listed in Table VII below.

PCR primer pairs were designed for each of the 11 genes encoding a gene product required for proliferation of Staphylococcus aureus as shown in Table VII. The upstream primers for each gene included the native ribosomal binding sites (S-D sequences). In addition, restriction sites for appropriate restriction enzymes were designed into the primers to facilitate directional cloning of the genes. PCR reactions were carried out using Pfu DNA polymerase (Stratagene, San Diego) under the following conditions per 50 u, l reaction: Pfu polymerase 2U, dNTP 200 AM, primers 400 nM each, S. aureus RN450 genomic DNA (template) 5-10 ng. The reaction involved an initial heating at 94 C for 5 min, followed by 25 cycles of 30 sec at 94 C/30 sec at 55 C/5 min at 72 C, and ending with 7 min of extension at 72 C.

The amplified genes were operably linked to the XylT5 promoter as follows. PCR products were cleaned using QIAGEN PCR Cleaning Kits and then were digested with the proper restriction enzymes. The resulting fragments were ligated overnight at 16 C with precut vector DNA containing the Xylits promoter. Ligation mixtures were ethanol precipitated at80 C for 20 min in the presence of 0.3 M sodium acetate. The precipitated DNA was spun down at 14,000 rpm for 30 min at 4 C and washed with 1 ml of 70% EtoH. The DNA pellets were air-dried and dissolved in EB or sterile water. To transform Staphylococcus aureus cells, the precipitated DNA was mixed with 45 ul of electroporation competent cells and incubated at room temperature for 30 min. The DNA/cell mixtures were electroporated (settings: 2 volts, 25 , uF, 200 Q) in 2 mm cuvettes and mixed with 450 gl B2 medium containing 0.2, ug/ml chloramphenicol. The cells were incubated at 37 C with shaking for 90 min. Transformed cells were plated onto LB agar plates containing chloramphenicol (34 ug/ml) for the selection of plasmids. Several colonies for each cloning reaction were picked and streaked to obtain a pure culture. Colony PCR reactions using vector-specific primers were performed to verify the size and identity of the inserts.

Gene-walking sequencing was employed to completely sequence the entire insert for several clones of each cloned gene. This was carried out to avoid using a cloned gene whose
DNA sequence was mutated during the PCR process.

To demonstrate that genes encoding gene products required for proliferation can confer resistance to their specific inhibitors upon induction at proper inducer levels, cells of each clone in which the genes were operably linked to the xylose inducible promoter were grown in LB medium with chloramphenicol (34 ug/ml) at a combination of differing antibiotic and inducer concentrations. This was accomplished by using microtitration plates (96 or 384 wells) which contained antibiotic and inducer at gradient concentrations in a matrix format in 10 times excess quantity (see Figure 11). Media containing inoculated cells (9 volume) was dispensed into the wells containing 1 volume of antibiotic/inducer for a final volume of 50, ul (for 384 well plates) or 200 ul (for 96 well plates). The plates were incubated at 37 C with periodic shaking and growth of cells was monitored by automatic measurement of optical density at OD600 using a Ultramark reader. A clone over-expressing a particular gene was considered resistant to its specific antibiotic (inhibitor) if significant growth was observed at appropriate inducer concentrations in the presence of a particular concentration of antibiotic but not in the absence of inducer at that concentration of antibiotic.

The results are indicated in Figure 12 and Figure 13. As illustrated in Figure 12, at appropriate concentrations of inducer cells which overexpress the defB gene product were able to grow at elevated concentrations of the antibiotic actinonin, which acts on the defB gene product. Similarly, as illustrated in Figure 13, at appropriate concentrations of inducer cells which overexpress the folA gene product were able to grow at elevated concentrations of the antibiotic trimethoprim, which acts on the folA gene product.

Thus, elevated expression of a gene product required for proliferation enables cells to grow in the presence of antibiotic concentrations which inhibit or prevent growth of wild type cells.

Table VII - Essential Genes/Proteins and Specific Inhibitors
EMI1574.1

EMI1575.1
* antibiotics unavailable commercially
EXAMPLE 22
Overexpression of Genes Encoding Gene Products Required for Proliferation Confers Specific
Resistance to Antibiotics which Target the Overexpressed Gene Product
To demonstrate that cells which overexpress a gene encoding a gene product required for proliferation are specifically resistant to antibiotics which target that gene product, the following experiments were performed. Several identical compound plates were prepared as described above in which different antibiotics were present in different wells. Media containing cells overexpressing different genes were separately dispensed into each one of these plates.

Plate incubation and growth measurement were the same as described in Example 21 above.

Growth was deemed specific if cells overexpressing one particular gene only gained resistance to antibiotics which target the product of the overexpressed gene but not to other antibiotics which target the products of genes which were not overexpressed.

As indicated in Figure 14 overexpression of the fabl gene conferred resistance to triclosan, which acts on the gene product of the fabI gene, enoyl-acyl carrier protein reductase.

However, overexpression of the fabI gene did not confer resistance to cerulenin, trimethoprim, or actinonin, each of which act on other gene products.

Similarly, as indicated in Figure 15 overexpression of the folA gene conferred resistance to trimethoprim, which acts on the gene product of the folA gene, dihydrofolate reductase.

However, overexpression of the folS gene did not confer resistance to triclosan, cerulenin, or actinonin, each of which act on other gene products.

As indicated in Figure 16 overexpression of the defB gene conferred resistance to actinonin, which acts on the gene product of the defB gene, peptide deformylase. However, overexpression of the defB gene did not confer resistance to cerulenin, trimethoprim, or triclosan, each of which act on other gene products.

As indicated in Figure 17 overexpression of the fabF gene conferred resistance to cerulenin, which acts on the gene product of the fabF gene, keto-acyl carrier protein synthase
II. However, overexpression of the fabF gene did not confer resistance to triclosan, trimethoprim, or actinonin, each of which act on other gene products.

Thus, overexpression of a gene encoding a gene product required for proliferation confers specific resistance to antibiotics which target the overexpressed gene product.

EXAMPLE 23
Selection of a Strain Overexpressing a Gene Encodiez a Target Gene Product from a Mixture of
Strains Overexpressing Genes Required for Proliferation
To confirm that a strain expressing the gene product targeted by an antibiotic can be selected from a mixture of strains which each overexpress a different

gene required for proliferation, the following experiment was performed. S. aureus strains overexpressing one of nine genes encoding a gene product required for proliferation were constructed as described above. The nine overexpressed genes were fabF, defB, folA, fabl, ileS, fusA, gyrB, murA, rpoB.

A mixture of the nine strains was grown wells in a 96 well plate in medium containing various concentrations of inducer and a sufficient concentration of actinonin, cerulenin, triclosan or trimethoprim to inhibit the growth of strains which do not overexpress the targets of these antibiotics.

Growth was observed in wells containing appropriate inducer concentrations and each one of the four antibiotics (See Figure 18). The cultures which grew in the presence of one of the antibiotics were analyzed as follows. The cultures were removed from the wells of the plate and single colonies were obtained by plating serial dilutions LB agar plates containing an appropriate antibiotic. Plasmids were isolated from at least 60 individual colonies for each culture and the genes which conferred antibiotic resistance were amplified by performing PCR reactions using vector-specific primers. The PCR products were then sequenced.

All of the plasmids obtained from the culture which grew in the presence of cerulenin contained the fabF sequence. Similarly, all of the plasmids obtained from clones which grew in the presence of triclosan contained the fabl gene. All of the plasmid obtained from colonies which grew in the presence of actinonin contained the defB gene. In addition, 81% of the plasmids obtained from colonies which grew in the presence of trimethoprim contained the folA gene. Growth conditions could be further optimized to provide 100% recovery of plasmids containing the folA gene.

These results demonstrate that a strain expressing the gene product targeted by an antibiotic can be selected from a mixture of strains which each overexpress a different gene required for proliferation.

EXAMPLE 24

Identification of Amplification Products Having Distinguishable Lengths

The following genes were identified as being required for proliferation as previously described in U. S. Patent Application Serial Number 09/815,242, filed march 21,2001.

Plasmids in which antisense nucleic acids complementary to nucleotide sequences the essential pbpC, secA, ylaO (Bs), yphC (Bs), trpS, polC, fabI, rpsR (Bs), fabF (yjaY), ileS, murC, fmhB, murA (Bs), murF (Bs), ftsZ, tufA, gyrA, rpoB, grlA or folA (dfrA) genes were transcribed from the XylT5 promoter in Staphylococcus aureus.

Amplification primers were designed which would yield amplification products of the following lengths if the plasmid encoding the corresponding antisense nucleic acid is present in a mixture of nucleic acids: yphC 260bp secA 267bp folA 230 bp tufA 243bp far 220bp gyrA 225bp trpS 208bp ileS 215bp fabF 189bp murs 203bp murA 176bp fmhB 181bp rpoB 159bp ylao 169bp grlA 151bp pbpC 156bp murC 129bp polC 145bp rpsR 109bp ftsZ 117bp

The 5'primer of each pair was complementary to a nucleotide sequence within the xy1T5 promoter while 3'primer was complementary to a nucleotide sequence within the antisense clone. The 5'primer of each pair was identical for each amplification reaction. The nucleotide sequence GTTTCTT was appended on the 5'end of the 3'primers. One primer in each pair was labeled with either VIC or 6FAM.

Two sets of ten plasmids containing the antisense nucleic acids complementary to the genes listed in each of the columns above were mixed in equal amounts in 11 tubes except that either the plasmid encoding antisense nucleic acids complementary to a nucleotide sequence in the grlA gene or the plasmid encoding antisense nucleic acids complementary to nucleotide sequences in the fmhB gene were serially diluted two fold in each of the 11 tubes (i. e. the first tube had 100pg of the grlA plasmid or the fmhB plasmid while the last tube had 0. 10pg of the grlA plasmid or the fmhB plasmid). Amplification reactions were conducted on the mixtures and the amplification products were separated on a 5% NuSieve 3: 1 agarose gel (BioWhittaker Molecular Applications Rockland, ME). The levels of the 151bp or 181 amplification products for the grIA or fmhB primer respectively were specifically reduced in a stepwise fashion with increasing dilutions while the levels of the undiluted products remained constant. The assay readily detected a 10-fold decrease in template concentration reflected in the amplification products corresponding to the grlA or fmhB plasmids.

Although this method has been described using examples of antisense nucleic acids to specific essential genes, it will be appreciated that this method can be used with any of the antisense nucleic acids described herein, such as an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, an antisense nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ ID
NOs.: 1-6213, a nucleic acid complementary to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of a nucleotide sequence selected from the group consisting of SEQ
ID NOs.: 6214-42397, a nucleic acid complementary to a nucleic acid which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID
NOs.: 42398-78581, a nucleic acid complementary to a nucleic acid which encodes at least 5, 10,15,20,25,30,35,40,50,75,100, or 150 consecutive amino acids of a polypeptide sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a homologous antisense nucleic acid, an antisense nucleic acid comprising at least 10,15,20,25,30,35,40,50,75,100, 150,200,300,400, or 500 consecutive nucleotides of a homologous nucleic acid, a nucleic acid complementary to a homologous coding nucleic acid, a nucleic acid complementary to at least 10,15,20,25,30,35,40,50,75,100,150,200,300,400, or 500 consecutive nucleotides of a homologous coding nucleic acid, a nucleic acid complementary to a nucleic acid which encodes a homologous polypeptide, or a nucleic acid complementary to a nucleic acid which encodes at least 5,10,15,20,25,30,35,40,50,75,100, or 150 consecutive amino acids of a homologous polypeptide. It will also be appreciated that promoters other than XlyT5 can be used to express the gene products described herein. For example, a number of promoters useful for nucleic acid expression (including antisense nucleic acid expression) in Enterococcus faecalis,
Staphylococcus areus as well as other Gram positive organisms are described in U. S. Patent
Application Serial Number 10/032,393, filed December 21,2001.

Additionally, the above methods can be used with any organism including
Acinetobacter baumannfi, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Candida albicans,
Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis,
Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis),
Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptheriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma p7leumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haefnolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimufiurn, Shigella boydii, Shigella dysenteriae,
Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis,
Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species.

EXAMPLE 25
Selective Disappearance of Amplification Products Corresponding to Strains Underexpressing a
Gene Product on which a Compound which Inhibits Proliferation Acts

Strains of Staphylococcus aureus containing plasmids encoding antisense nucleic acids complementary to nucleotide sequences within the yphC, folA, fall, trpS, fabF, murA, rpoB, grlA, murC or rpsR genes (described in Example 24 above) were mixed together in identical cultures such that the number of cells of each strain in the culture was identical. Each of the cultures containing the ten strains was contacted with one of the following antibiotics at one of the following concentrations : spectinomycin-2.5,5.0ug/ml mupriocin-4.3,8.6,17.2ug/ml. cerulenin-4.5,9.0,18. Oug/ml
Spectinomycin acts on the product of the rpsR gene, mupriocin acts on the product of the ileS gene and cerulenin acts on the product of the FabF gene. The middle concentration for each antibiotic is its IC50.

The culture containing the ten strains were grown in rich medium (L-Broth; for antisense LB + chloroamphenicol to maintain antisense plasmid) until the cells reached early log phase then contacted with of one of the above-stated compounds at one of the concentrations listed above (preferably near IC50). The cultures were grown for a sufficient length of time to permit the compounds to specifically inhibit the growth of strains underexpressing their targets. Preferably the cultures were grown at least 16 hr, more preferably between 24 and 48 hrs. It is desirable to avoid allowing the culture to grow for time periods which might places selective pressure on the strains which could lead to false positives.

The cells were harvested by centrifugation and plasmid DNA was isolated from the cultures. PCR amplifications were performed as described in Example 24. Amplification products were run on NuSieve agarose gels as described above. The amounts of the amplification products corresponding to each antisense nucleic acid were determined and compared to those in a control culture which was not contacted with the drug or to the amounts of the amplification products corresponding to the other antisense nucleic acids which were not complementary to nucleotide sequences in the genes encoding the gene products on which the compounds act. In each case, only the amplification product corresponding to the target on which the antibiotic acts was not detectable on the gel.

It is desirable, in embodiments in which the level or activity of gene products is regulated by transcribing antisense nucleic acids complementary to gene products required for proliferation or by replacing the native promoters of such genes with regulatable promoters, to perform dose-response curve for the inducer used to induce transcription of the antisense nucleic acids or induce transcription from the regulatable promoter. In such embodiments, it is desirable to use the lowest concentration of inducer which provides optimal transcription levels for detecting the effects of a particular test compound while interfering as little as possible with the growth of strains which do not overexpress or underexpress the target on which the compound acts. It also desirable contact the cultures with varying amounts of test compounds to determine the optimal amounts for obtaining differential growth of strains which overexpress or underexpress the targets on which the compounds act. Preferably, if the strains overexpress gene products required for proliferation, the level of the compound is preferably about ICgo or above. Preferably, if the strains underexpress gene products required for proliferation, the level of the compound is preferably about ICso or below.

It will be appreciated that, if desired, the amplification products may be detected using the dyes described above. It will also be appreciated that amplification products may be detected using any desired amplification method including RT-PCR and PCR.

Although this method has been described using examples of antisense nucleic acids to specific essential genes, it will be appreciated that this method can be used with any of the antisense nucleic acids described herein, such as an antisense nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 1-6213, a nucleic acid sequence complementary to a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NOs.: 6214-42397, a nucleic acid sequence complementary to a nucleic acid sequence which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs.: 42398-78581, a homologous antisense nucleic acid, a nucleic acid sequence complementary to a homologous coding nucleic acid, or a nucleic acid complementary to a nucleic acid which encodes a homologous polypeptide. It will also be appreciated that promoters other than XlyT5 can be used to express the gene products described herein. For example, a number of promoters useful for nucleic acid expression (including antisense nucleic acid expression) in Enterococcus faecalis, Staplaylococcus areus as well as other Gram positive organisms are described in U. S. Patent Application Serial Number 10/032, 393, filed December 21,2001.

Additionally, the above methods can be used with any organism including

Acinetobacter baumannii, Anaplasma marginale, Aspergillus fumigatus, Bacillus anthracis,
Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia,
Burkholderia futagorum, Burkholderia mallei, Cathpylobacter jejuni, Candida albicans,
Candida glabrata (also called Torulopsis glabrata), Candida tropicalis, Candida parapsilosis,
Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis),
Candida dubliniensis, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens,
Coccidioides immitis, Corynebacterium diptlteriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum, Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium,
Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica, Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseurlornonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae,
Shigella flexneri, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis,
Staphylococcus haemolyticus, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, within vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species.

EXAMPLE 26

Use of Identified Nucleic Acid Sequences as Probes

The sequences from Escherichia coli, Staphylococcus aureus, Enterococcusfaecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni,
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinuni, Clostridium difficile, Corynebacteriulli diptheriae, Enterobacter cloacae,
Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis described herein, homologous coding nucleic acids, or homologous antisense nucleic acids can be used as probes to obtain the sequence of additional genes of interest from a second cell or microorganism. For example, probes to genes encoding potential bacterial target proteins may be hybridized to nucleic acids from other organisms including other bacteria and higher organisms, to identify homologous sequences in these other organisms. For example, the identified sequences from Escherichia coli,
Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Coryunebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida,

Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae,
Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or
Yersinia pestis, homologous coding nucleic acids, or homologous antisense nucleic acids may be used to identify homologous sequences in Acinetobacter baumannii, Anaplasma marginale,
Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei,
Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata),
Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium , friizgens, Co cidioides immi is Co nebacterium dipt eriae, Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium,
Escherichia coli, Haemophilus influenzae, Helicobacter pylori, Histoplasma capsulatum,
Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica,
Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the nucleic acids from
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baurnannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis described herein, homologous coding nucleic acids, or homologous antisense nucleic acids may be used to identify homologous nucleic acids from a heterologous organism other than E. coli.

Hybridization between the nucleic acids from Escherichia coli Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei Campylobacter jejuni Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,

Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi Salmonella typhi Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis described herein, homologous coding nucleic acids, or homologous antisense nucleic acids and nucleic acids from humans might indicate that the protein encoded by the gene to which the probe corresponds is found in humans and therefore not necessarily an optimal drug target. Alternatively, the gene can be conserved only in bacteria and therefore would be a good drug target for a broad spectrum antibiotic or antimicrobial. These probes can also be used in a known manner to isolate homologous nucleic acids from Staphylococcus, Salmonella, Klebsiella, Pseudomonas,
Enterococcus or other cells or microorganisms, e. g. by screening a genomic or cDNA library.

Probes derived from the nucleic acid sequences from Escherichia coli Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi
Burkholderia cepacia, Burkholderia fungorum, Burkholderia naallei, Carnpylobacter jejuni, Chlarnydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrl2alis, Myeobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi Salmonella typhi Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis described herein, homologous coding nucleic acids, or homologous antisense nucleic acids, or portions thereof, can be labeled with detectable labels familiar to those skilled in the art, including radioisotopes and non-radioactive labels, to provide a detectable probe. The detectable probe can be single stranded or double stranded and can be made using techniques known in the art, including in vitro transcription, nick translation, or kinase reactions. A nucleic acid sample containing a sequence capable of hybridizing to the labeled probe is contacted with the labeled probe. If the nucleic acid in the sample is double stranded, it can be denatured prior to contacting the probe. In some applications, the nucleic acid sample can be immobilized on a surface such as a nitrocellulose or nylon membrane. The nucleic acid sample can comprise nucleic acids obtained from a variety of sources, including genomic DNA, cDNA libraries, RNA, or tissue samples.

Procedures used to detect the presence of nucleic acids capable of hybridizing to the detectable probe include well known techniques such as Southern blotting, Northern blotting, dot blotting, colony hybridization, and plaque hybridization. In some applications, the nucleic acid capable of hybridizing to the labeled probe can be cloned into vectors such as expression vectors, sequencing vectors, or in vitro transcription vectors to facilitate the characterization and expression of the hybridizing nucleic acids in the sample. For example, such techniques can be used to isolate, purify and clone sequences from a genomic library, made from a variety of bacterial species, which are capable of hybridizing to probes made from the sequences identified as decribed herein.

EXAMPLE 27
Preparation of PCR Primers and Amplification of DNA
The identified Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei Campylobacter jejuni Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae,
Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,

Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis genes corresponding directly to or located within the operon of nucleic acid sequences required for proliferation, homologous coding nucleic acids, or homologous antisense nucleic acids or portions thereof can be used to prepare PCR primers for a variety of applications, including the identification or isolation of homologous sequences from other species. For example, the Escherichia coli, Staphylococcus aureus, Enterococcus faecalis,
Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus artthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi,
Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei Campylobacter jejuni
Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae,
Enterococcus faeciunt, Haemophilus influenzae, Helicobacter pylori Legionella pneumophila,
Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis genes may be used to prepare PCR primers to identify or isolate homologous sequences from Acinetobacter baumannii, Anaplasma marginale,
Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei
Campylobacter jejuni Candida albicans, Candida glabrata (also called Torulopsis glabrata),
Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Co7ynebacterium diptheriae,
Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium,
Escherichia coli Haemophilus influenzae, Helicobacter pylori Histoplasma capsulatum,
Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica,
Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi Salmonella typhi, Salmonella typhimurium, Shigella boydii Shigella dysenteriae, Shigella flexneri Shigella sonnei
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutans, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibriio cholerae, Vibrio parahaemolyticus, Vibriio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the PCR primers may be used to identify or isolate homologous nucleic acids from an organism other than E. coli.

The identified or isolated nucleic acids obtained using the PCR primers may contain part or all of the homologous nucleic acids. Because homologous nucleic acids are related but not identical in sequence, those skilled in the art will often employ degenerate sequence PCR primers.

Such degenerate sequence primers are designed based on sequence regions that are either known to be conserved or suspected to be conserved such as conserved coding regions. The successful production of a PCR product using degenerate probes generated from the sequences identified herein would indicate the presence of a homologous gene sequence in the species being screened.

The PCR primers are at least 10 nucleotides, and preferably at least 20 nucleotides in length. More preferably, the PCR primers are at least 20-30 nucleotides in length. In some embodiments, the
PCR primers can be more than 30 nucleotides in length. It is preferred that the primer pairs have approximately the same G/C ratio, so that melting temperatures are approximately the same. A variety of PCR techniques are familiar to those skilled in the art. For a review of PCR technology, see Molecular Cloning to Genetic Engineering White, B. A. Ed. in Methods in Molecular Biology 67: Humana Press, Totowa 1997. When the entire coding sequence of the target gene is known, the 5'and 3'regions of the target gene can be used as the sequence source for PCR probe generation. In each of these PCR procedures, PCR primers on either side of the nucleic acid sequences to be amplified are added to a suitably prepared nucleic acid sample along with dNTPs and a thermostable polymerase such as Taq polymerase, Pfu polymerase, or Vent polymerase. The nucleic acid in the sample is denatured and the PCR primers are specifically hybridized to complementary nucleic acid sequences in the sample. The hybridized primers are extended.

Thereafter, another cycle of denaturation, hybridization, and extension is initiated. The cycles are repeated multiple times to produce an amplified fragment containing the nucleic acid sequence between the primer sites.

EXAMPLE 28
Inverse PCR
The technique of inverse polymerase chain reaction can be used to extend the known nucleic acid sequence identified as described herein. The inverse PCR reaction is described generally by Ochman et al., in Ch. 10 of PCR Technology: Principles and Applications for
DNA Amplification, (Henry A. Erlich, Ed.) W. H. Freeman and Co. (1992). Traditional PCR requires two primers that are used to prime the synthesis of complementary strands of DNA. In inverse PCR, only a core sequence need be known.

Using the sequences identified as relevant from the techniques taught in Examples 10 and 11 and applied to other species of bacteria, a subset of nucleic sequences are identified that correspond to genes or operons that are required for bacterial proliferation. In species for which a genome sequence is not known, the technique of inverse PCR provides a method for obtaining the gene in order to determine the sequence or to place the probe sequences in full context to the target sequence to which the identified nucleic acid sequence binds.

To practice this technique, the genome of the target organism is digested with an appropriate restriction enzyme so as to create fragments of nucleic acid that contain the identified sequence as well as unknown sequences that flank the identified sequence. These fragments are then circularized and become the template for the PCR reaction. PCR primers are designed in accordance with the teachings of Example 27 and directed to the ends of the identified sequence..

The primers direct nucleic acid synthesis away from the known sequence and toward the unknown sequence contained within the circularized template. After the PCR reaction is complete, the resulting PCR products can be sequenced so as to extend the sequence of the identified gene past the core sequence of the identified exogenous nucleic acid sequence identified. In this manner, the full sequence of each novel gene can be identified. Additionally the sequences of adjacent coding and noncoding regions can be identified.

EXAMPLE 29
Identification of Genes Required for Escherichia coli Proliferation
Genes required for proliferation in Escherichia coli are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 30

Identification of Genes Required for Staphylococcus aureus Proliferation

Genes required for proliferation in Staphylococcus aureus are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 31

Identification of Genes Required for Enterococcus faecalis Proliferation

Genes required for proliferation in Enterococcus faecalis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 32

Identification of Genes Required for Klebsiella pneumoniae Proliferation

Genes required for proliferation in Klebsiella pneumoniae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 33

Identification of Genes Required for Pseudomonas aeruzinosa Proliferation

Genes required for proliferation in Pseudomonas aeruginosa are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 34

Identification of Genes Required for Salmonella tvphimurium Proliferation

Genes required for proliferation in Sal77lonella typhimurium are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 35

Identification of Genes Required for Acinetobacter baumannii Proliferation

Genes required for proliferation in Acinetobacter baumannii are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 36

Identification of Genes Required for Bacillus anthracis Proliferation

Genes required for proliferation in Bacillus anthracis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 37

Identification of Genes Required for Bordetella pertussis Proliferation

Genes required for proliferation in Bordetella pertussis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 38

Identification of Genes Required for Borrelia burgdorferi Proliferation

Genes required for proliferation in Borrelia burgdorferi are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 39

Identification of Genes Required for Burkholderia cepacia Proliferation

Genes required for proliferation in Burkholderia cepacia are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 40

Identification of Genes Required for Burkholderia funvorum Proliferation

Genes required for proliferation in Burkholderia fungorum are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 41

Identification of Genes Required for Burkholderia mallei Proliferation

Genes required for proliferation in Burkholderia mallei are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 42

Identification of Genes Required for Campvlobacter Lejuni Proliferation

Genes required for proliferation in Campylobacter jejuni are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 43

Identification of Genes Required for Chlamvdia pneumoniae Proliferation

Genes required for proliferation in Chlamydia pneumoniae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 44

Identification of Genes Required for Chlamvdia trachomatis Proliferation

Genes required for proliferation in Chlamydia trachomatis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 45

Identification of Genes Required for Clostridium acetobutvlicum Proliferation

Genes required for proliferation in Clostridium acetobutylicum are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 46

Identification of Genes Required for Clostridium botulinuna Proliferation

Genes required for proliferation in Clostridium botulinum are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 47

Identification of Genes Required for Clostridium difficile Proliferation

Genes required for proliferation in Clostridium difficile are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 48

Identification of Genes Required for Corvnebacterium diptheriae Proliferation

Genes required for proliferation in Corynebacterium diptheriae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 49

Identification of Genes Required for Enterobacter cloacae Proliferation

Genes required for proliferation in Enterobacter cloacae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 50

Identification of Genes Required for Enterococcus faecium Proliferation

Genes required for proliferation in Enterococcus faecium are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 51

Identification of Genes Required for Haemophilus influenzae Proliferation

Genes required for proliferation in Haemophilus influenzae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 52

Identification of Genes Required for Helicobacter pvlori Proliferation

Genes required for proliferation in Helicobacter pylori are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 53

Identification of Genes Required for Legionella pneumophila Proliferation

Genes required for proliferation in Legionella pneumophila are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 54

Identification of Genes Required for Listeria monoctogenes Proliferation

Genes required for proliferation in Listeria monocytogenes are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 55

Identification of Genes Required for Moraxella catarrhalis Proliferation

Genes required for proliferation in Moraxella catarrhalis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 56

Identification of Genes Required for M I ti

Genes required for proliferation in Mycobacterium avium are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 57

Identification of Genes Required for M

Genes required for proliferation in Mycobacterium bovis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 58

Identification of Genes Required for Mycobacterium leprae Proliferation

Genes required for proliferation in Mycobacterium leprae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 59

Identification of Genes Required for Mveobacterium tuberculosis Proliferation

Genes required for proliferation in Mycobacterium tuberculosis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 60

Identification of Genes Required for M

Genes required for proliferation in Mycoplasma genitalium are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 61

Identification of Genes Required for Mveoplasma pneumoniae Proliferation

Genes required for proliferation in Mycoplasma pneumoniae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 62

Identification of Genes Required for Neisseria gonorrhoeae Proliferation

Genes required for proliferation in Neisseria gonorrhoeae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 63

Identification of Genes Required for Neisseria fneningitidis Proliferation

Genes required for proliferation in Neisseria meningitidis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 64

Identification of Genes Required for Pasteurella multocida Proliferation

Genes required for proliferation in Pasteurella multocida are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 65

Identification of Genes Required for Proteus mirabilis Proliferation

Genes required for proliferation in Proteus mirabilis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 66

Identification of Genes Required for Pseudomonas putida Proliferation

Genes required for proliferation in Pseudomonas putida are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 67

Identification of Genes Required for Pseudomonas syringae Proliferation

Genes required for proliferation in Pseudomonas syringae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 68

Identification of Genes Required for Salmonella paratyphi Proliferation

Genes required for proliferation in Salmonella paratyphi are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 69

Identification of Genes Required for Salmonella typhi Proliferation

Genes required for proliferation in Salmonella typhi are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 70

Identification of Genes Required for Staphvlococcus e, nidernzidis Proliferation

Genes required for proliferation in Staphylococcus epidermidis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 71

Identification of Genes Required for Staphvlococcus haemol ticus Proliferation

Genes required for proliferation in Staphylococcus haemolyticus are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 72

Identification of Genes Required for Streptococcus mutans Proliferation

Genes required for proliferation in Streptococcus mutans are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 73

Identification of Genes Required for Streptococcus pneumoniae Proliferation

Genes required for proliferation in Streptococcus pneumoniae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 74

Identification of Genes Required for Streptococcus pyogenes Proliferation

Genes required for proliferation in Streptococcus pyogenes are identified according to the methods described above. For example, promoters and vectors

described herein can be used to identify essential genes described herein.

EXAMPLE 75

Identification of Genes Required for Treponema pallidum Proliferation

Genes required for proliferation in Treponema pallidum are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 76

Identification of Genes Required for Ureaplas za urealvticum Proliferation

Genes required for proliferation in Ureaplasma urealyticum are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 77

Identification of Genes Required for Vibrio cholerae Proliferation

Genes required for proliferation in Yibrio cholerae are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 78

Identification of Genes Required for Yersinia pestis Proliferation

Genes required for proliferation in Yersinia pestis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 79

Identification of Genes Required for Salmonella enterica Proliferation

Genes required for proliferation in Salmonella enterica are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 80

Identification of Genes Required for Aspergillus fumigatus Proliferation

Genes required for proliferation in Aspergillus fumigatus are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 81

Identification of Genes Required for Plasmodium ovale Proliferation

Genes required for proliferation in Plasmodium ovale are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 82

Identification of Genes Required for Entamoeba histolvtica Proliferation

Genes required for proliferation in Entamoeba histolytica are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 83

Identification of Genes Required for Candida albicans Proliferation

Genes required for proliferation in Candida albicans are identified according to the methods described above. For example, promoters and vectors described

herein can be used to identify essential genes described herein.

EXAMPLE 84

Identification of Genes Required for Histoplasma capsulatum Proliferation

Genes required for proliferation in Histoplasma capsulatum are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

EXAMPLE 85

Identification of Genes Required for Salmonella cholerasuis Proliferation

Genes required for proliferation in Salmonella cholerasuis are identified according to the methods described above. For example, promoters and vectors described herein can be used to identify essential genes described herein.

Use of Isolated Exogenous Nucleic Acid Fragments as Antisense Antibiotics

In addition to using the identified sequences to enable screening of molecule libraries to identify compounds useful to identify antibiotics, antisense nucleic acids complementary to the proliferation-required sequences or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids, or homologous antisense nucleic acids can be used as therapeutic agents. Specifically, the proliferation-required sequences or homolgous coding nucleic acids, or portions therof, in an antisense orientation or homologous antisense nucleic acids can be provided to an individual to inhibit the translation of a bacterial target gene or the processing, folding, or assembly into a protein/RNA complex of a nontranslated RNA.

EXAMPLE 86

Generation of Antisense Therapeutics from Identified Exogenous Sequences

Antisense nucleic acids complementary to the proliferation-required sequences described herein, or portions thereof, antisense nucleic acids complementary to homologous coding nucleic acids, or portions thereof, or homologous antisense nucleic acids or portions thereof can be used as antisense therapeutics for the treatment of bacterial infections or simply for inhibition of bacterial growth in vitro or in vivo. For example, the antisense therapeutics may be used to treat bacterial infections caused by Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baunaannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes,Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or to inhibit the growth of these organisms. The antisense therapeutics may also be used to treat infections caused by or to inhibit the growth of Acinetobacter baumannii, Anaplasma marginale,
Aspergillus fumigatus, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei,
Campylobacter jejuni, Candida albicans, Candida glabrata (also called Torulopsis glabrata),
Candida tropicalis, Candida parapsilosis, Candida guilliermondii, Candida krusei, Candida kefyr (also called Candida pseudotropicalis), Candida dubliniensis, Chlamydia pneumoniae,
Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Coccidioides immitis, Corynebacterium diptheriae,
Cryptococcus neoformans, Enterobacter cloacae, Enterococcus faecalis, Enterococcus faecium,

Escherichia coli, Haen2ophilus influefzzae, Helicobacter pylori, Histoplasma capsulatum,
Klebsiella pneumoniae, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Nocardia asteroides, Pasteurella haemolytica,
Pasteurella multocida, Pneumocystis carinii, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas putida, Pseudomonas syringae, Salmonella bongori, Salmonella cholerasuis, Salmonella enterica, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shigella boydii, Shigella dysenteriae, Shigella flexneri, Shigella sonnei,
Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus haemolyticus,
Streptococcus pneumoniae, Streptococcus mutants, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Vibrio parahaemolyticus, Vibrio vulnificans, Yersinia enterocolitica, Yersinia pestis or any species falling within the genera of any of the above species. In some embodiments of the present invention, the antisense therapuetics may be used to treat infection by or inhibit the growth of an organism other than E. coli.

The therapy exploits the biological process in cells where genes are transcribed into messenger RNA (mRNA) that is then translated into proteins. Antisense RNA technology contemplates the use of antisense nucleic acids, including antisense oligonucleotides, complementary to a target gene that will bind to its target nucleic acid and decrease or inhibit the expression of the target gene. For example, the antisense nucleic acid may inhibit the translation or transcription of the target nucleic acid. In one embodiment, antisense oligonucleotides can be used to treat and control a bacterial infection of a cell culture containing a population of desired cells contaminated with bacteria. In another embodiment, the antisense oligonucleotides can be used to treat an organism with a bacterial infection.

Antisense oligonucleotides can be synthesized from any of the sequences of the present invention using methods well known in the art. In a preferred embodiment, antisense oligonucleotides are synthesized using artificial means. Uhlmann & Peymann, Chemical Rev.

90: 543-584 (1990) review antisense oligonucleotide technology in detail. Modified or unmodified antisense oligonucleotides can be used as therapeutic agents. Modified antisense oligonucleotides are preferred. Modification of the phosphate backbones of the antisense oligonucleotides can be achieved by substituting the internucleotide phosphate residues with methylphosphonates, phosphorothioates, phosphoramidates, and phosphate esters.

Nonphosphate internucleotide analogs such as siloxane bridges, carbonate brides, thioester bridges, as well as many others known in the art may also be used. The preparation of certain antisense oligonucleotides with modified internucleotide linkages is described in U. S. Patent
No. 5,142,047.

Modifications to the nucleoside units of the antisense oligonucleotides are also contemplated. These modifications can increase the half-life and increase cellular rates of uptake for the oligonucleotides in vivo. For example, a-anomeric nucleotide units and modified nucleotides such as 1,2-dideoxy-d-ribofuranose, 1,2-dideoxy-1-phenylribofuranose, and N4, N4- ethano-5-methyl-cytosine are contemplated for use in the present invention.

An additional form of modified antisense molecules is found in peptide nucleic acids.

Peptide nucleic acids (PNA) have been developed to hybridize to single and double stranded nucleic acids. PNA are nucleic acid analogs in which the entire deoxyribose-phosphate backbone has been exchanged with a chemically different, but structurally homologous, polyamide (peptide) backbone containing 2-aminoethyl glycine units. Unlike DNA, which is highly negatively charged, the PNA backbone is neutral. Therefore, there is much less repulsive energy between complementary strands in a PNA-DNA hybrid than in the comparable DNA-DNA hybrid, and consequently they are much more stable. PNA can hybridize to DNA in either a Watson/Crick or
Hoogsteen fashion (Demidov et al., Proc. Natl. Acad. Sci. US. A. 92: 2637-2641,1995; Egholm,
Nature 365: 566-568, 1993; Nielsen et al., Science 254: 1497-1500,1991; Dueholm et al., New J.

Chem. 21: 19-31,1997).

Molecules called PNA"clamps"have been synthesized which have two identical PNA sequences joined by a flexible hairpin linker containing three 8-amino-3,6-dioxaoctanoic acid units.

When a PNA clamp is mixed with a complementary homopurine or homopyrimidine DNA target sequence, a PNA-DNA-PNA triplex hybrid can form which has been shown to be extremely stable (Bentin et al., Biochemistry 35: 8863-8869, 1996; Egholm et al., Nucleic Acids Res. 23: 217-222, 1995; Griffith et al., J. Am. Chem. Soc. 117: 831-832,1995).

The sequence-specific and high affinity duplex and triplex binding of PNA have been extensively described (Nielsen et al., Science 254: 1497-1500,1991; Egholm et al., J. Am. Chem.

Soc. 114: 9677-9678,1992; Egholm et al., Nature 365: 566-568, 1993; Almarsson et al., Proc. Natl.

Acad. Sci. US. A. 90: 9542-9546,1993; Demidov et al., Proc. Natl. Acad. Sci. US. A. 92: 2637-2641, 1995). They have also been shown to be resistant to nuclease and protease digestion (Demidov et al., Biochem. Pharm. 48: 1010-1313,1994). PNA has been used to inhibit gene expression (Hanvey et al., Science 258: 1481-1485,1992; Nielsen et al., Nucl. Acids. Res., 21: 197-200,1993;
Nielsen et al., Gene 149: 139-145,1994; Good & Nielsen, Science, 95: 2073-2076,1998; to block restriction enzyme activity (Nielsen et al., supra., 1993), to act as an artificial transcription promoter (Mollegaard, Proc. Natl. Acad. Sci. U. S. A. 91: 3892-3895, 1994) and as a pseudo restriction endonuclease (Demidov et al., Nucl. Acids. Res. 21: 2103-2107,1993). Recently, PNA has also been shown to have antiviral and antitumoral activity mediated through an antisense mechanism (Norton, Nature Biotechnol., 14: 615-619,1996; Hirschman et al., S : Investig. Med.

44: 347-351,1996). PNAs have been linked to various peptides in order to promote PNA entry into cells (Basu et al., Bioconj*. Chein. 8: 481-488,1997; Pardridge et al., Proc. Natl. Acad. Sci. U. S. A.

92:5592-5596,1995).

The antisense oligonucleotides contemplated by the present invention can be administered by direct application of oligonucleotides to a target using standard techniques well known in the art. The antisense oligonucleotides can be generated within the target using a plasmid, or a phage. Alternatively, the antisense nucleic acid may be expressed from a sequence in the chromosome of the target cell. For example, a promoter may be introduced into the chromosome of the target cell near the target gene such that the promoter directs the transcription of the antisense nucleic acid. Alternatively, a nucleic acid containing the antisense sequence operably linked to a promoter may be introduced into the chromosome of the target cell. It is further contemplated that the antisense oligonucleotides are incorporated in a ribozyme sequence to enable the antisense to specifically bind and cleave its target mRNA. For technical applications of ribozyme and antisense oligonucleotides see Rossi et al., Pharmacol. Ther.

50 (2): 245-254, (1991). The present invention also contemplates using a retron to introduce an antisense oligonucleotide to a cell. Retron technology is exemplified by U. S. Patent No.

5,405,775. Antisense oligonucleotides can also be delivered using liposomes or by electroporation techniques which are well known in the art.

The antisense nucleic acids described above can also be used to design antibiotic compounds comprising nucleic acids which function by intracellular triple helix formation. Triple helix oligonucleotides are used to inhibit transcription from a genome. The antisense nucleic acids can be used to inhibit cell or microorganism gene expression in individuals infected with such microorganisms or containing such cells. Traditionally, homopurine sequences were considered the most useful for triple helix strategies. However, homopyrimidine sequences can also inhibit gene expression. Such homopyrimidine

oligonucleotides bind to the major groove at homopurine : homopyrimidine sequences. Thus, both types of sequences based on the sequences from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneu7710niae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or homologous nucleic acids that are required for proliferation are contemplated for use as antibiotic compound templates.

The antisense nucleic acids, such as antisense oligonucleotides, which are complementary to the proliferation-required nucleic acids from Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneujraophila, Listeria monocytogeraes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae,
Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae,
Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or
Yersinia pestis or to homologous coding nucleic acids, or portions thereof, may be used to induce bacterial cell death or at least bacterial stasis by inhibiting target nucleic acid transcription or translation. Antisense oligonucleotides complementary to about 8 to 40 nucleotides of the proliferation-required nucleic acids described herein or homologous coding nucleic acids have sufficient complementarity to form a duplex with the target sequence under physiological conditions.

To kill bacterial cells or inhibit their growth, the antisense oligonucleotides are applied to the bacteria or to the target cells under conditions that facilitate their uptake. These conditions include sufficient incubation times of cells and oligonucleotides so that the antisense oligonucleotides are taken up by the cells. In one embodiment, an incubation period of 7-10 days is sufficient to kill bacteria in a sample. An optimum concentration of antisense oligonucleotides is selected for use.

The concentration of antisense oligonucleotides to be used can vary depending on the type of bacteria sought to be controlled, the nature of the antisense oligonucleotide to be used, and the relative toxicity of the antisense oligonucleotide to the desired cells in the treated culture. Antisense oligonucleotides can be introduced to cell samples at a number of different concentrations preferably between lx10-l M to lxl04M. Once the minimum concentration that can adequately control gene expression is identified, the optimized dose is translated into a dosage suitable for use in vivo. For example, an inhibiting concentration in culture of lx10-7 translates into a dose of approximately 0.6 mg/kg body weight. Levels of oligonucleotide approaching 100 mg/kg body weight or higher may be possible after testing the toxicity of the oligonucleotide in laboratory animals. It is additionally contemplated that cells from the subject are removed, treated with the antisense oligonucleotide, and reintroduced into the subject. This range is merely illustrative and one of skill in the art are able to determine the optimal concentration to be used in a given case.

After the bacterial cells have been killed or controlled in a desired culture, the desired cell population may be used for other purposes.

EXAMPLE 87

Use of Antisense Oligonucleotides to Treat Contaminated Cell Cultures

The following example demonstrates the ability of an Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlarnydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium ddcile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis antisense oligonucleotide or an antisense oligonucleotide complementary to a homologous coding nucleic acid, or portions thereof, to act as a bacteriocidal or bacteriostatic agent to treat a contaminated cell culture system. The application of the antisense oligonucleotides of the present invention are thought to inhibit the translation of bacterial gene products required for proliferation. The antisense nucleic acids may also inhibit the transcription, folding or processing of the target
RNA.

In one embodiment of the present invention, the antisense oligonucleotide may comprise a phosphorothioate modified nucleic acid comprising at least about 15, at least about 20, at least about 25, at least about 30, at least about 35, at least about 40, or more than 40 consecutive nucleotides of an antisense nucleic acid listed in Table IA (SEQ ID NOs.: 1-6213).

A sense oligodeoxynucleotide complementary to the antisense sequence is synthesized and used as a control. The oligonucleotides are synthesized and purified according to the procedures of
Matsukura, et al., Gene 72: 343 (1988). The test oligonucleotides are dissolved in a small volume of autoclaved water and added to culture medium to make a 100 micromolar stock solution.

Human bone marrow cells are obtained from the peripheral blood of two patients and cultured according standard procedures well known in the art. The culture is contaminated with
Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae,
Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderiamallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum,
Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis,
Mycobacterium leprae, Mycobacterium tuberculosis, Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans,
Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or an organism containing a homologous nucleic acid and incubated at 37 C overnight to establish bacterial infection.

The control and antisense oligonucleotide containing solutions are added to the contaminated cultures and monitored for bacterial growth. After a 10 hour incubation of culture and oligonucleotides, samples from the control and experimental cultures are drawn and analyzed for the translation of the target bacterial gene using standard microbiological techniques well known in the art. The target Escherichia coli, Staphylococcus aureus,

Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannEi, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis gene or an organism containing the homologous coding nucleic acid is found to be translated in the control culture treated with the control oligonucleotide, however, translation of the target gene in the experimental culture treated with the antisense oligonucleotide of the present invention is not detected or reduced, indicating that the culture is no longer contaminated or is contaminated at a reduced level.

EXAMPLE 88
Use of Antisense Oligonucleotides to Treat Infections
A subject suffering from a Escherichia coli Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium,
Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis,
Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei,
Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonor7 hoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Yersinia pestis infection or an infection with an organism containing a homologous coding nucleic acid is treated with the antisense oligonucleotide preparation above. The antisense oligonucleotide is provided in a pharmaceutically acceptable carrier at a concentration effective to inhibit the transcription or translation of the target nucleic acid. The present subject is treated with a concentration of antisense oligonucleotide sufficient to achieve a blood concentration of about 0.1-100 micromolar. The patient receives daily injections of antisense oligonucleotide to maintain this concentration for a period of 1 week. At the end of the week a blood sample is drawn and analyzed for the presence or absence of the organism using standard techniques well known in the art. There is no detectable evidence of Escherichia coli, Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumanfz. ii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptlaeriae, Enterobacter cloacae, Enterococcus faecium, Haemophilus influenzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epidermidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Yibrio cholerae, Yersinia pestis or an organim

containing a homologous coding nucleic acid and the treatment is terminated.

Antisense nucleic acids complementary to a homologous coding nucleic acid or a portion thereof may be used in the preceding method to treat individuals infected with an organism containing the homologous coding nucleic acid.

EXAMPLE 89

Preparation and Use of Triple Helix Forming Oligonucleotides

The sequences of proliferation-required nucleic acids, homologous coding nucleic acids, or homologous antisense nucleic acids are scanned to identify 10-mer to 20-mer homopyrimidine or homopurine stretches that could be used in triple-helix based strategies for inhibiting gene expression. Following identification of candidate homopyrimidine or homopurine stretches, their efficiency in inhibiting gene expression is assessed by introducing varying amounts of oligonucleotides containing the candidate sequences into a population of bacterial cells that normally express the target gene. The oligonucleotides may be prepared on an oligonucleotide synthesizer or they may be purchased commercially from a company specializing in custom oligonucleotide synthesis.

The oligonucleotides can be introduced into the cells using a variety of methods known to those skilled in the art, including but not limited to calcium phosphate precipitation, DEAE

Dextran, electroporation, liposome-mediated transfection or native uptake.

Treated cells are monitored for a reduction in proliferation using techniques such as monitoring growth levels as compared to untreated cells using optical density measurements. The oligonucleotides that are effective in inhibiting gene expression in cultured cells can then be introduced in vivo using the techniques well known in that art at a dosage level shown to be effective.

In some embodiments, the natural (beta) anomers of the oligonucleotide units can be replaced with alpha anomers to render the oligonucleotide more resistant to nucleases. Further, an intercalating agent such as ethidium bromide, or the like, can be attached to the 3'end of the alpha oligonucleotide to stabilize the triple helix. For information on the generation of oligonucleotides suitable for triple helix formation see Griffin et al. (Science 245: 967-971 (1989), which is hereby incorporated by this reference).

EXAMPLE 90

Identification of Bacterial Strains from Isolated Specimens by PCR

Classical bacteriological methods for the detection of various bacterial species are time consuming and costly. These methods include growing the bacteria isolated from a subject in specialized medium, cultivation on selective agar medium, followed by a set of confirmation assays that can take from 8 to 10 days or longer to complete. Use of the identified sequences of the present invention provides a method to dramatically reduce the time necessary to detect and identify specific bacterial species present in a sample.

In one exemplary method, bacteria are grown in enriched medium and DNA samples are isolated from specimens of, for example, blood, urine, stool, saliva or central nervous system fluid by conventional methods. A panel of PCR primers based on identified sequences unique to various species or types of cells or microorganisms are then utilized in accordance with Example 27 to amplify DNA of approximately 100-200 nucleotides in length from the specimen. A separate PCR reaction is set up for each pair of PCR primers and after the PCR reaction is complete, the reaction mixtures are assayed for the presence of PCR product. The presence or absence of bacteria from the species to which the PCR primer pairs belong is determined by the presence or absence of a PCR product in the various test PCR reaction tubes.

Although the PCR reaction is used to assay the isolated sample for the presence of various bacterial species, other assays such as the Southern blot hybridization are also contemplated.

Compounds which inhibit the activity or reduce the amount of gene products required for proliferation may be identified using rational drug design. These methods may be used with the proliferation-required polypeptides described herein or homologous polypeptides. In such methods, the structure of the gene

product is determined using methods such as x-ray crystallography, NMR, or computer modelling. Compounds are screened to identify those which have a structure which allows them to interact with the gene product. In some embodiments, the compounds are screened to identify those which have structures which allow them to interact with regions of the gene product which are important for its activity. For example, the compounds may be screened to identify those which have structures which allow them to bind to the active site of the gene product to inhibit its activity. For example, the compound may be a suicide substrate which binds to the active site with high affinity, thereby preventing the gene product from acting on its natural substrate. Alternatively, the compound may bind to a region of the gene product which is involved in complex formation with other biomolecules. In such instances, the activity of the gene product is inhibited by blocking the interaction between the gene product and other members of the complex.

Thus, one embodiment of the present invention comprises a method of using a crystal of the gene products of the present invention and/or a dataset comprising the three-dimensional coordinates obtained from the crystal in a drug-screening assay. The present invention also includes agents (modulators or drugs) that are identified by the methods of the present invention, along with the method of using agents (modulators or drugs) identified by a method of the present invention, for inhibiting the activity of or modulating the amount of an essential gene product. The present invention also includes crystals comprising the gene products of the present invention or portions thereof.

In some embodiments of the present invention, the three-dimensional structure of the polypeptides required for proliferation is determined using X-ray crystallography or NMR. The coordinates of the determined structure are used in computer-assisted modeling programs to identify compounds that bind to and/or modulate the activity or amount of the encoded polypeptide. The method may include the following steps: 1) the generation of high-purity crystals of the encoded recombinant (or endogenous) polypeptide for analysis; 2) determination of the three-dimensional structure of the polypeptide; and, 3) the use of computer-assisted "docking"programs to analyze the molecular interaction of compound structure and the polypeptide (i. e., drug screening).

General methods for performing each of the above steps are described below and are also well known to those of skill in the art. Any method known to those of skill in the art, including those described herein, may be employed for generating the three-dimensional structure for each identified essential gene product and its use in the drug-screening assays.

Crystals of the gene products required for proliferation may be obtained as follows.

Under certain conditions, molecules condense from solution into a highly-ordered crystalline lattice, which is defined by a unit cell, the smallest repeating volume of the crystalline array.

The contents of such a cell can interact with and diffract certain electromagnetic and particle waves (e. g., X-rays, neutron beams, electron beams etc.). Due to the symmetry of the lattice, the diffracted waves interact to create a diffraction pattern. By measuring the diffraction pattern, crystallographers are able to reconstruct the three-dimensional structure of the atoms in the crystal.

Any method known to those of skill in the art, including those set forth below, may be employed to prepare high-purity crystals. For example, crystals of the product of the identified essential gene can be grown by a number of techniques including batch crystallization, vapor diffusion (either by sitting drop or hanging drop) and by microdialysis. Seeding of the crystals in some instances is required to obtain X-ray quality crystals. Standard micro and/or macro seeding of crystals may therefore be used. Exemplified below is the hanging-drop vapor diffusion procedure. Hanging drops of an essential gene product (2.5 ul, 10 mg/ml) in 20 mM
Tris, pH=8.0,100 mM NaCI are mixed with an equal amount of reservoir buffer containing 2.73.2 M sodium formate and 100 mM Tris buffer, pH=8. 0, and kept at 4 C. Crystal showers may appear after 1-2 days with large single crystals growing to full size (0.3 X 0.3 X 0.15 mm3) within 2-3 weeks. Crystals are harvested in 3.5 M sodium formate and 100 mM Tris buffer, pH=8.0 and cryoprotected in 3.5 M sodium formate, 100 mM Tris buffer, pH=8. 0,10% (w/v) sucrose, and 10% (v/v) ethylene glycol before flash freezing in liquid propane. In some embodiments, the crystal may be obtained using the methods described in U. S. Patent No.

5,869,604. The method involves (a) contacting a mixture containing uncrystallized polypeptides with an exogenous nucleating agent that has an areal lattice match of at least 90.4% to the polypeptide, (b) crystallizing the polypeptides, thereby forming at least one crystal of the polypeptide attached to the nucleating agent, the attached crystal being of a high purity, and at least one polypeptide crystal unattached to the nucleating agent, the unattached crystal being of a lower purity than the attached crystal, and (c) separating the crystal attached to the nucleating agent from the crystal unattached to the nucleating agent. The crystallized polypeptide may also be purified from contaminants by (a) contacting a mixture containing uncrystallized polypeptides and a contaminant with an exogenous nucleating agent that has an areal lattice match of at least 90.4% to the polypeptide, (b) crystallizing the polypeptides, thereby forming at least one crystal of the polypeptide attached to the nucleating agent, the attached crystal being of a high purity and produced in a high yield, and at least one crystal unattached to the nucleating agent, the unattached crystal being of a lower purity than the attached crystal, and (c) separating the crystal attached to the nucleating agent from the crystal unattached to the nucleating agent.

Once a crystal of the present invention is grown, X-ray diffraction data can be collected using methods familiar to those skilled in the art. Therefore, any person with skill in the art of protein crystallization having the present teachings and without undue experimentation can crystallize a large number of alternative forms of the essential gene products from a variety of different organisms, or polypeptides having conservative substitutions in their amino acid sequence.

A crystal lattice is defined by the symmetry of its unit cell and any structural motifs the unit cell contains. For example, there are 230 possible symmetry groups for an arbitrary crystal lattice, while the unit cell of the crystal lattice group may have an arbitrary dimension that depends on the molecules making up the lattice. Biological macromolecules, however, have asymmetric centers and are limited to 65 of the 230 symmetry groups. See Cantor et al., Biophysical Chemistry, Vol. III, W. H. Freeman & Company (1980).

A crystal lattice interacts with electromagnetic or particle waves, such as X-rays or electron beams respectively, that have a wavelength with the same order of magnitude as the spacing between atoms in the unit cell. The diffracted waves are measured as an array of spots on a detection surface positioned adjacent to the crystal. Each spot has a three-dimensional position, hld, and an intensity, I (hkl), both of which are used to reconstruct the threedimensional electron density of the crystal with the so-called Electron Density Equation. The
Electron Density Equation states that the three-dimensional electron density of the unit cell is the Fourier transform of the structure factors. Thus, in theory, if the structure factors are known for a sufficient number of spots in the detection space, then the three-dimensional electron density of the unit cell could be calculated using the Electron Density Equation.

In some embodiments of the present invention, an image of a crystal of a gene product required for proliferation or a portion thereof is obtained with the aid of a digital computer and the crystal's diffraction pattern as described in U. S. Patent No. 5,353,236. The diffraction pattern contains a plurality of reflections, each having an associated resolution. The image is obtained by (a) converting the diffraction pattern of the crystal into computer usable normalized amplitudes, the pattern being produced with a diffractometer ; (b) determining from the diffraction pattern a dimension of a unit cell of the crystal; (c) providing an envelope defining the region of the unit cell occupied by the gene product or portion thereof in the crystal; (d) distributing a collection of scattering bodies within said envelope, the collection of scattering bodies having various arrangements, each of which has an associated pattern of Fourier amplitudes; (e) condensing the collection of scattering bodies to a condensed arrangement that results in a high correlation between a diffraction pattern and the pattern of Fourier amplitudes for said collection of scattering bodies; (f) determining the phase associated with at least one of the reflections of said diffraction pattern from the condensed arrangement of scattering bodies; (g) calculating an electron density distribution of the gene product or portion thereof within the unit cell from the phase determined in procedure f; and (h) displaying a graphical image of the gene product or portion thereof constructed from said electron density distribution.

The crystals of the gene products required for proliferation may be used in drug screening methods such as those described in U. S. Patent Number 6,156,526. Briefly, in such methods, a compound which inhibits the formation of a complex comprising the gene product or a portion thereof is identified as follows. A set of atomic coordinates defining the threedimensional structure of a complex including the gene product of interest or a portion thereof are determined. A potential compound that binds to the gene product or a portion thereof involved in complex formation is selected using the atomic coordinates obtained

above. The compound is contacted with the gene product or portion thereof and its binding partner (s) in the complex under conditions which would permit the complex to form in the absence of the potential compound. The binding affinity of the gene product or portion thereof for its binding partner (s) is determined and a potential compound is identified as a compound that inhibits the formation of the complex when there is a decrease in the binding affinity of the gene product or portion thereof for its binding partner (s).

In some embodiments of the present invention, the three dimensional structure of the essential gene product is determined and potential agonists and/or potential antagonists are designed with the aid of computer modeling [Bugg et al., Scientific American, Dec.: 92-98 (1993); West et al., TIPS, 16: 67-74 (1995); Dunbrack et al., Folding & Design, 2: 27-42 (1997).

Computer analysis may be performed with one or more of the computer programs including: QUANTA, CHARMM, INSIGHT, SYBYL, MACROMODEL and ICM [Dunbrack et al., Folding & Design, 2: 27-42 (1997)].. In a further embodiment of this aspect of the invention, an initial drug-screening assay is performed using the three-dimensional structure so obtained, preferably along with a docking computer program. Such computer modeling can be performed with one or more Docking programs such as FlexX, DOC, GRAM and AUTO
DOCK [Dunbrack et al., Folding & Design, 2: 27-42 (1997)].

It should be understood that for each drug screening assay provided herein, a number of iterative cycles of any or all of the steps may be performed to optimize the selection. The drug screening assays of the present invention may use any of a number of means for determining the interaction between an agent or drug and an essential gene product.

* In some embodiments of the present invention, a drug can be specifically designed to bind to an essential gene product of the present invention through NMR based methodology.

[Shuker et al., pi Science 274: 1531-1534 (1996)]. NMR spectra may be recorded using devices familiar to those skilled in the art, such as the Varian Unity Plus 500 and unity 600 spectrometers, each equipped with a pulsed-field gradient triple resonance probe as analyzed as described in Bagby et al., [Cell 82: 857-867 (1995)]. Sequential resonance assignments of backbone'H,.'5 N, and. C atoms may be made using a combination of triple resonance experiments similar to those previously described [Bagby et al., Biochemistry, 33: 2409-2421 (1994a), except with enhanced sensitivity [Muhandiram and Kay, J. Magn. Reson., 103: 203216 (1994), and minimal H20 saturation [Kay et al., J. Magn. Reson., 109: 129-133 (1994)].

Side chain'H and 13 C assignments may be made using HCCH-TOCSY [Bax et al., J. Magn.

Reson., 87: 620-627 (1990)] experiments with mixing times of 8 ms and 16 ms. in solution but need not be included in structure calculations. Nuclear Overhauser effect (NOE) cross peaks in two-dimensional'H--'H NOE spectroscopy (NOESY), three-dimensional 15 N-edited NOESY
HSQC [Zhang et al., J. Biomol, NMR, 4: 845-858 (1994)] and three-dimensional simultaneous acquisition lS N/'3C-edited NOE [Pascal et al., J. Magn. Reson., 103: 197-201 (1994)], spectra may be obtained with 100 ms NOE mixing times. Standard pseudo-atom distance corrections [Wuthrich et al., J. Mol. Biol., 169: 949-961 (1983)], may be incorporated to account for center averaging. An additional 0.5. ANG. may be added to the upper limits for distances involving methyl groups [Wagner et al., J. Mol. Biol., 196: 611-639 (1987) ; Clore et al., Biochemistry, 26: 8012-8023 (1987)].

The structures can be calculated using a simulated annealing protocol [Nilges et al., In computational Aspects of the Study of Biological Macromolecules by Nuclear Magnetic
Resonance Spectroscopy, J. C. Hoch, F. M. Poulsen, and C. Redfield, eds., New York: Plenum
Press, pp. 451-455 (1991)], within X-PLOR [Brunger, X-PLOR Manual, Version 3.1, New
Haven, Conn.: Department of Molecular Biophysics and Biochemistry, Yale University (1993)], using the previously described strategy [Bagby et al., Structure, 2: 107-122 (1994b)].

Interhelical anges may be calculated using a program written by K. Yap. Accessible surface areas were calculated using the program Naccess, available from

Prof. J. Thornton, University
College, London.

Compounds capable of reducing the activity or amount of gene products required for cellular proliferation may be identified using the methods described in US Pat. No. 6,077,682.

Briefly, the three-dimensional structure of the gene product or portion thereof may be used in a drug screening assay by (a) selecting a potential drug by performing rational drug design with the three-dimensional structure determined from one or more sets of atomic coordinates of the gene product or portion thereof in conjunction with computer modeling; (b) contacting the potential drug with a polypeptide comprising the gene product or portion thereof and (c) detecting the binding of the potential drug with said polypeptide; wherein a potential drug is selected as a drug if the potential drug binds to the polypeptide. In some methods, the threedimensional structure of the gene product or portion thereof is used in a drug screening assay involving (a) selecting a potential drug by performing structural based rotational drug design with the three-dimensional structure of the gene product or portion thereof; wherein said selecting is performed in conjunction with computer modeling; (b) contacting the potential drug with a polypeptide comprising the gene product or portion thereof in the presence of a substrate of the gene product; wherein in the absence of the potential drug the substrate is acted upon by the gene product; and (c) determining the extent to which the gene product acted upon the substrate; wherein a drug is selected when a decrease in the action of the gene product on the substrate is determined in the presence of the potential drug relative to in its absence. In some embodiments, the preceding method further involves (d) contacting the potential drug with the gene product or portion thereof for NMR analysis; wherein a binding complex forms between the potential drug and said gene product or portion thereof for NMR analysis; wherein the gene product or portion thereof for NMR analysis comprises a conservative amino acid substitution; (e) determining the three-dimensional structure of the binding complex by NMR; and (f) selecting a candidate drug by performing structural based rational drug design with the threedimensional structure determined for the binding complex; wherein said selecting is performed in conjunction with computer modeling; (g) contacting the candidate drug with a second polypeptide comprising the gene product or portion thereof in the presence of a substrate of the gene product or portion thereof; wherein in the absence of the candidate drug the substrate is acted upon by the second polypeptide; and (h) determining the amount of action of the second polypeptide on the substrate; wherein a drug is selected when a decrease in the amount of action of the second polypeptide is determined in the presence of the candidate drug relative to in its absence.

Once the three-dimensional structure of a crystal comprising an essential gene product is determined, a potential modulator of its activity, can be examined through the use of computer modeling using a docking program such as FlexX, GRAM, DOCK, or AUTODOCK [Dunbrack et al., 1997, supra], to identify potential modulators. This procedure can include computer fitting of potential modulators to the polypeptide or fragments thereof to ascertain how well the shape and the chemical structure of the potential modulator will bind. Computer programs can also be employed to estimate the attraction, repulsion, and steric hindrance of the two binding partners (e. g., the essential gene product and a potential modulator). Generally the tighter the fit, the lower the steric hindrances, and the greater the attractive forces, the more potent the potential modulator since these properties are consistent with a tighter binding constant. Furthermore, the more specificity in the design of a potential drug the more likely that the drug will not interact as well with other proteins. This will minimize potential side-effects due to unwanted interactions with other proteins.

Compound and compound analogs can be systematically modified by computer modeling programs until one or more promising potential analogs is identified. In addition systematic modification of selected analogs can then be systematically modified by computer modeling programs until one or more potential analogs are identified. Such analysis has been shown to be effective in the development of HIV protease inhibitors [Lam et al., Science 263: 380-384 (1994) ; Wlodawer et al., Ann. Rev. Biochem. 62: 543-585 (1993) ; Appelt,
Perspectives in Drug Discovery and Design 1: 23-48 (1993) ; Erickson, Perspectives in Drug
Discovery and Design 1: 109-128 (1993)]. Alternatively a potential modulator could be obtained by initially screening a random peptide library produced by recombinant bacteriophage for example, [Scott and Smith, Science, 249: 386-390 (1990); Cwirla et al., Proc. Natl. Acad.

Sci., 87: 6378-6382 (1990); Devlin et al., Science, 249: 404-406 (1990)]. A peptide selected in this manner would then be systematically modified by computer modeling programs as described above, and then treated analogously to a structural analog.

Example 91 describes computer modelling of the structures of gene products required for proliferation.

EXAMPLE 91
Determination of the Structure of Gene Products Required for Proliferation Using Computer Modelling
Three dimensional models were built by applying computer modelling methods to some of the gene products required for proliferation of Staphylococcus aureus using the amino acid sequences of the encoded proteins as follows. Sir Tom Blundell's program COMPOSER as provided by Tripos Associates in their BIOPOLYMER module to SYBYL was used to build the models. Skolnik's method of topology fingerprinting as implemented in Matchmaker was used to score the average mutation free energy. This number is in Boltzmans (units of kT) and should be negative (the more negative, the better the model.

Composer uses a Needleman Wunsch alignment with jumbling to find significant alignments. The reported parameters are percent identity and significance as measured from the jumbling. Those matches which were 30% identical and had a significance greater that 4 on the scale were judged to be good candidates for model building templates. If no three dimensional structures met these criteria, then a BLAST search was conducted against the most recent PDB sequence database. Any significant hits discovered in this manner were then added to the binary protein structure database and the candidate search was repeated in the manner discussed above.

In the next phase, Composer assigned structurally conserved and structurally variable regions and built the backbone structure and then searched the database for structures of the variable loops. These were then spliced in and a model of the protein resulted. Any loops (variable regions) which were unassignable were manually built and refined with a combination of dynamics.

The structure was then refined. Hydrogen atoms were added and a non-active aggregate was defined. 1000pS of dynamics using AMBER ALL-ATOM and Kollman charges are performed. Next a minimization cycle of up 5000 steepest decent steps were performed and then the aggregate was thawed and the process was repeated on the entire protein.

The resulting structure was then validated in MATCHMAKER. The topologicaly scanned free energy determined from empirically derived protein topologies was computed and the average energy/residue is reported in Boltzamans was reported. As this number represents a free energy the more negative it is the more favorable it is.

Sixty six proteins required for the proliferation of Staphylococcus aureus were modelled as described above. MATCHMAKER energies were computed for these. The distribution of the models built by class is shown in Table VIM below.

Table VIII : Distribution of models built with their MATCHMAKER energies in kT
EMI1615.1

Classification	Number of Models	Average Matchmaker Energy
Acylases	1	-0. 10
Dehydrogenases	3	-0. 12
DNA Related	3	-0. 12

Heat Shock Protein 2-0. 16

Hydrolases 3 -0.16

Isomerases 1 0.05

Ligases 7-0. 07

Lyases 1-0. 09

Membrane Anchored 1-0. 12

Misc 18-0. 21

Oxidoreductases 6-0. 09

Proteases 1-0. 03

Ribosome 3-0. 11

Synthases 4-0.14

Transferases 6-0.12

The validity of the above method was confirmed using FtsZ. In the case of FtsZ, a crystal structure from M. Janeschi was available. Examination of the gross structural features determined using the above modelling showed all of the folds in the correct place, although there were some minor differences from the structure determined by x-ray crystallography.

EXAMPLE 92

Functional Complementation

In another embodiment, gene products whose activities may be complemented by a proliferation-required gene product from Escherichia coli, Staphylococcus aureus,
Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Acinetobacter baumannii, Bacillus anthracis, Bacteroides fragilis, Bordetella pertussis, Borrelia burgdorferi, Burkholderia cepacia, Burkholderia fungorum, Burkholderia mallei, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Clostridium acetobutylicum, Clostridium botulinum, Clostridium difficile, Corynebacterium diptheriae,
Enterobacter cloacae, Enterococcus faecium, Haemophilus influe7lzae, Helicobacter pylori,
Legionella pneumophila, Listeria monocytogenes, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium leprae, Mycobacterium tuberculosis,
Mycoplasma genitalium, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Proteus mirabilis, Pseudomonas putida, Pseudomonas syringae, Salmonella paratyphi, Salmonella typhi, Staphylococcus epiderrnidis, Staphylococcus haemolyticus, Streptococcus mutans, Streptococcus pheumoniae, Streptococcus pyogenes,
Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae or Yersinia pestis or homologous polypeptides are identified using merodiploids, created by introducing a plasmid or

Bacterial Artificial Chromosome into an organism having a mutation in the essential gene which reduces or eliminates the activity of the gene product. In some embodiments, the mutation may be a conditional mutation, such as a temperature sensitive mutation, such that the organism proliferates under permissive conditions but is unable to proliferate under nonpermissive conditions in the absence of complementation by the gene on the plasmid or Bacterial Artificial Chromosome. Alternatively, duplications may be constructed as described in Roth et al. (1987) Biosynthesis of Aromatic Amino Acids in Escherichia coli and Salmonella typhimurium, F. C. Neidltardt, ed., American Society for Microbiology, publisher, pp. 22692270. Such methods are familiar to those skilled in the art.

It will be appreciated that no matter how detailed the foregoing appears in text, the invention can be practiced in many ways. As is also stated above, it should further be noted that the use of particular terminology when describing certain features or aspects of the present invention should not be taken to imply that the broadest reasonable meaning of such terminology is not intended, or that the terminology is being re-defined herein to be restricted to including any specific characteristics of the features or aspects of the invention with which that terminology is associated. Thus, although this invention has been described in terms of certain preferred embodiments, other embodiments which will be apparent to those of ordinary skill in the art in view of the disclosure herein are also within the scope of this invention. Accordingly, the scope of the invention is intended to be defined only by reference to the appended claims and any equivalents thereof.

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